COMPOUNDS, COMPOSITIONS, AND METHODS

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application number 60/468,744, filed May 7, 2003, which is incorporated herein by reference for all purposes.

FIELD OF THE INVENTION

[0002] This invention relates to compounds which are inhibitors of the mitotic kinesin KSP and are useful in the treatment of cellular proliferative diseases, for example cancer, hyperplasias, restenosis, cardiac hypertrophy, immune disorders, fungal disorders, and inflammation.

BACKGROUND OF THE INVENTION

[0003] Among the therapeutic agents used to treat cancer are the taxanes and vinca alkaloids, which act on microtubules. Microtubules are the primary structural element of the mitotic spindle. The mitotic spindle is responsible for distribution of replicate copies of the genome to each of the two daughter cells that result from cell division. It is presumed that disruption of the mitotic spindle by these drugs results in inhibition of cancer cell division, and induction of cancer cell death. However, microtubules form other types of cellular structures, including tracks for intracellular transport in nerve processes. Because these agents do not specifically target mitotic spindles, they have side effects that limit their usefulness.

[0004] Improvements in the specificity of agents used to treat cancer is of considerable interest because of the therapeutic benefits which would be realized if the side effects associated with the administration of these agents could be reduced. Traditionally, dramatic improvements in the treatment of cancer are associated with identification of therapeutic agents acting through novel mechanisms. Examples of this include not only the taxanes, but also the camptothecin class of topoisomerase I inhibitors. From both of these perspectives, mitotic kinesins are attractive targets for new anti-cancer agents.

[0005] Mitotic kinesins are enzymes essential for assembly and function of the mitotic spindle, but are not generally part of other microtubule structures, such as in nerve processes. Mitotic kinesins play essential roles during all phases of mitosis. These enzymes are "molecular motors" that transform energy released by hydrolysis of ATP into mechanical force which drives the directional movement of cellular cargoes along microtubules. The catalytic domain sufficient for this task is a compact structure of approximately 340 amino acids. During mitosis, kinesins organize microtubules into the bipolar structure that is the mitotic spindle. Kinesins mediate movement of chromosomes along spindle microtubules, as well as structural changes in the mitotic spindle associated with specific phases of mitosis. Experimental perturbation of mitotic kinesin function causes malformation or dysfunction of the mitotic spindle, frequently resulting in cell cycle arrest and cell death.

[0006] Among the mitotic kinesins which have been identified is KSP. KSP belongs to an evolutionarily conserved kinesin subfamily of plus end-directed microtubule motors that assemble into bipolar homotetramers consisting of antiparallel homodimers. During mitosis KSP associates with microtubules of the mitotic spindle. Microinjection of antibodies directed against KSP into human cells prevents spindle pole separation during prometaphase, giving rise to monopolar spindles and causing mitotic arrest and induction of programmed cell death. KSP and related kinesins in other, non-human, organisms, bundle antiparallel microtubules and slide them relative to one another, thus forcing the two spindle poles apart. KSP may also mediate in anaphase B spindle elongation and focussing of microtubules at the spindle pole.

[0007] Human KSP (also termed HsEg5) has been described (Blangy, et al., Cell, 83:1159-69 (1995); Whitehead, et al., Arthritis Rheum., 39:1635-42 (1996); Galgio et al., J. Cell Biol., 135:339-414 (1996); Blangy, et al., J Biol. Chem., 272:19418-24 (1997); Blangy, et al., Cell Motil Cytoskeleton, 40:174-82 (1998); Whitehead and Rattner, J. Cell Sci., 111:2551-61 (1998); Kaiser, et al., JBC 274:18925-31 (1999); GenBank accession numbers: X85137, NM004523 and U37426), and a fragment of the KSP gene (TRIP5) has been described (Lee, et al., Mol Endocrinol., 9:243-54 (1995); GenBank accession number L40372). Xenopus KSP homologs (Eg5), as well as Drosophila KLP61 F/KRP130 have been reported.

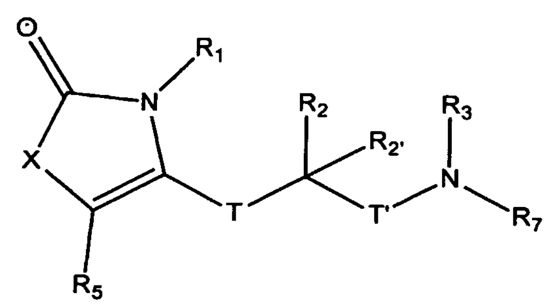
[0008] Mitotic kinesins, including KSP, are attractive targets for the discovery

and development of novel antimitotic chemotherapeutics. Accordingly, it is an object of the present invention to provide compounds, compositions and methods useful in the inhibition of KSP.

SUMMARY OF THE INVENTION

[0009] In accordance with the objects outlined above, the present invention provides compounds that can be used to treat cellular proliferative diseases. The compounds are KSP inhibitors. The present invention also provides compositions comprising such compounds, and methods utilizing such compounds or compositions, which can be used to treat cellular proliferative diseases.

[0010] In one aspect, the invention relates to methods for treating cellular proliferative diseases, and for treating disorders by inhibiting the activity of KSP. The methods employ one or more compounds represented by Formula I:



Formula I

wherein:

T and T' are independently a covalent bond or optionally substituted lower alkylene;

X is O or $-NR_4$;

R_1 is hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, or optionally substituted heteroaralkyl-;

R_2 and $R_{2'}$ are independently hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, or optionally substituted heteroaralkyl; or R_2 and $R_{2'}$ taken together form an optionally

substituted 3- to 7-membered ring which optionally incorporates from one to two heteroatoms, selected from N, O, and S in the ring

R_3 is hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, optionally substituted heteroaralkyl-, -C(O)-R_6, or -S(O)$_2$-R_{6a};

R_5 is hydrogen, halogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, or optionally substituted heteroaralkyl-;

R_4 is hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, or optionally substituted heteroaralkyl-; or R_4 and R_5 taken together with the carbon and nitrogen to which they are bound, respectively, form an optionally substituted 5- to 7-membered ring;

R_6 is hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, optionally substituted heteroaralkyl, R_9O- or R_{11}-NH-;

R_{6a} is optionally substituted alkyl, optionally substituted aryl, optionally substituted alkylaryl, optionally substituted heteroaryl, optionally substituted alkylheteroaryl, or R_{11}-NH-;

R_7 is hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, or optionally substituted heteroaralkyl;

or R_7 taken together with R_3, and the nitrogen to which they are bound, form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates from one to two additional heteroatoms, chosen from N, O, and S in the heterocycle ring;

or R_7 taken together with R_2 form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates from one to two additional heteroatoms, chosen from N, O, and S in the heterocycle ring;

R_9 is optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, or optionally substituted heteroaralkyl and

R_{11} is hydrogen, optionally substituted alkyl, optionally substituted aryl,

optionally substituted aralkyl, optionally substituted heteroaryl, or optionally substituted heteroaralkyl;

(Formula I including single stereoisomers and mixtures of stereoisomers);

a pharmaceutically acceptable salt of a compound of Formula I;

a pharmaceutically acceptable solvate of a compound of Formula I; or

a pharmaceutically acceptable solvate of a pharmaceutically acceptable salt of a compound of Formula I.

[0011] In one aspect, the invention relates to methods for treating cellular proliferative diseases and other disorders that can be treated by inhibiting KSP by the administration of a therapeutically effective amount of a compound of Formula I; a pharmaceutically acceptable salt of a compound of Formula I; a pharmaceutically acceptable solvate of a compound of Formula I; or a pharmaceutically acceptable solvate of a pharmaceutically acceptable salt of a compound of Formula I. Such diseases and disorders include cancer, hyperplasia, restenosis, cardiac hypertrophy, immune disorders, fungal disorders and inflammation.

[0012] In another aspect, the invention relates to compounds useful in inhibiting KSP kinesin. The compounds have the structures shown above in Formula I; a pharmaceutically acceptable salt of a compound of Formula I; a pharmaceutically acceptable solvate of a compound of Formula I; or a pharmaceutically acceptable solvate of a pharmaceutically acceptable salt of a compound of Formula I. The invention also relates to pharmaceutical compositions comprising: a therapeutically effective amount of a compound of Formula I; a pharmaceutically acceptable salt of a compound of Formula I; a pharmaceutically acceptable solvate of a compound of Formula I; or a pharmaceutically acceptable solvate of a pharmaceutically acceptable salt of a compound of Formula I; and one or more pharmaceutical excipients. In another aspect, the composition further comprises a chemotherapeutic agent other than a compound of the present invention.

[0013] In an additional aspect, the present invention provides methods of screening for compounds that will bind to a KSP kinesin, for example compounds that will displace or compete with the binding of a compound of the invention. The methods comprise combining a labeled compound of the invention, a KSP kinesin, and at least one candidate agent and determining the binding of the candidate agent to the KSP kinesin.

[0014] In a further aspect, the invention provides methods of screening for modulators of KSP kinesin activity. The methods comprise combining a compound of the invention, a KSP kinesin, and at least one candidate agent and determining the effect of the candidate agent on the KSP kinesin activity.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0015] As used in the present specification, the following words and phrases are generally intended to have the meanings as set forth below, except to the extent that the context in which they are used indicates otherwise. The following abbreviations and terms have the indicated meanings throughout

Ac	=	acetyl
Aq	=	aqueous
Bn	=	benzyl
Boc	=	t-butyloxy carbonyl
Bu	=	butyl
c-	=	cyclo
CBZ	=	carbobenzoxy = benzyloxycarbonyl
DCM	=	dichloromethane = methylene chloride = CH_2Cl_2
DIEA	=	DIPEA = N,N-diisopropylethylamine
DMF	=	N,N-dimethylformamide
DMSO	=	dimethyl sulfoxide
Et	=	ethyl
h or hr	=	hour
HOAc	=	acetic acid
Me	=	methyl
min	=	minute
Ms	=	methanesulfonyl = mesyl
Ph	=	phenyl
Py	=	pyridine
rt	=	room temperature
sat'd	=	saturated
s-	=	secondary

t-	=	tertiary
TFA	=	trifluoroacetic acid
THF	=	tetrahydrofuran
Ts	=	tosylate

[0016] **Alkyl** is intended to include linear, branched, or cyclic aliphatic hydrocarbon structures and combinations thereof, which structures can be saturated or unsaturated. **Lower-alkyl** refers to alkyl groups of from 1 to 5 carbon atoms, preferably from 1 to 4 carbon atoms. Examples of lower-alkyl groups include methyl-, ethyl-, propyl-, isopropyl-, butyl-, s-and t-butyl and the like. Preferred alkyl groups are those of C_{20} or below. More preferred alkyl groups are those of C_{13} or below. **Cycloalkyl** is a subset of alkyl and includes cyclic aliphatic hydrocarbon groups of from 3 to 13 carbon atoms. Examples of cycloalkyl groups include c- propyl-, c-butyl-, c-pentyl-, norbornyl-, adamantyl and the like. **Cycloalkyl-alkyl-** is another subset of alkyl and refers to cycloalkyl attached to the parent structure through a non-cyclic alkyl-. Examples of cycloalkyl-alkyl- include cyclohexylmethyl-, cyclopropylmethyl-, cyclohexylpropyl-, and the like. In this application, alkyl includes alkanyl-, alkenyl and alkynyl residues; it is intended to include vinyl-, allyl-, isoprenyl and the like. When an alkyl residue having a specific number of carbons is named, all geometric isomers having that number of carbons are intended to be encompassed; thus, for example, "butyl" is meant to include n-butyl-, sec-butyl-, isobutyl and t-butyl-; "propyl" includes n-propyl-, isopropyl-, and c-propyl-.

[0017] **Alkylene-, alkenylene-, and alkynylene-** are other subsets of alkyl-, including the same residues as alkyl-, but having two points of attachment within a chemical structure. Examples of alkylene include ethylene ($-CH_2CH_2-$), propylene ($-CH_2CH_2CH_2-$), dimethylpropylene ($-CH_2C(CH_3)_2CH_2-$) and cyclohexylpropylene ($-CH_2CH_2CH(C_6H_{13})-$). Likewise, examples of alkenylene include ethenylene ($-CH=CH-$), propenylene ($-CH=CH-CH_2-$), and cyclohexylpropenylene ($-CH=CHCH(C_6H_{13})-$). Examples of alkynylene include ethynylene ($-C\equiv C-$) and propynylene ($-CH\equiv CH-CH_2-$).

[0018] **Cycloalkenyl** is a subset of alkyl and includes unsaturated cyclic hydrocarbon groups of from 3 to 13 carbon atoms. Examples of cycloalkenyl groups include c-hexenyl-, c-pentenyl and the like.

[0019] **Alkoxy** or **alkoxyl** refers to an alkyl group, preferably including from 1 to 8 carbon atoms, of a straight, branched, or cyclic configuration, or a combination thereof, attached to the parent structure through an oxygen (i.e., the group alkyl-O-). Examples include methoxy-, ethoxy-, propoxy-, isopropoxy-, cyclopropyloxy-, cyclohexyloxy- and the like. **Lower-alkoxy** refers to alkoxy groups containing one to four carbons.

[0020] **Acyl** refers to groups of from 1 to 8 carbon atoms of a straight, branched, or cyclic configuration or a combination thereof, attached to the parent structure through a carbonyl functionality. Such groups may be saturated or unsaturated, and aliphatic or aromatic. One or more carbons in the acyl residue can be replaced by oxygen, nitrogen (e.g., carboxamido), or sulfur as long as the point of attachment to the parent remains at the carbonyl. Examples include acetyl-, benzoyl-, propionyl-, isobutyryl-, oxalyl-, t-butoxycarbonyl-, benzyloxycarbonyl, morpholinylcarbonyl, and the like. **Lower-acyl** refers to acyl groups containing one to four carbons.

[0021] **Amino** refers to the group $-NH_2$. The term "substituted amino" refers to the group -NHR or -NRR where each R is independently chosen from the group: optionally substituted alkyl-, optionally substituted alkoxy, optionally substituted aminocarbonyl-, optionally substituted aryl-, optionally substituted heteroaryl-, optionally substituted heterocyclyl-, acyl-, alkoxycarbonyl-, sulfanyl-, sulfinyl and sulfonyl-, e.g., diethylamino, methylsulfonylamino, furanyl-oxy-sulfonamino. Substituted amino includes the groups $–NR^cCOR^b$, $–NR^cCO_2R^a$, and $-NR^cCONR^bR^c$, where

R^a is an optionally substituted C_1-C_6 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group;

R^b is H or optionally substituted C_1-C_6 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group; and

R^c is hydrogen or C_1-C_4 alkyl-; and

where each optionally substituted R^b group is independently unsubstituted or substituted with one or more substituents independently chosen from C_1-C_4 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, heteroaryl-C_1-C_4 alkyl-, C_1-C_4 haloalkyl-, $-OC_1$-C_4 alkyl, $-OC_1$-C_4 alkylphenyl, $-C_1$-C_4 alkyl-OH, $-OC_1$-C_4 haloalkyl, halogen, -OH, $-NH_2$, $-C_1$-C_4 alkyl-NH_2, $-N(C_1$-C_4 alkyl)(C_1-C_4 alkyl), $-NH(C_1$-C_4 alkyl),

-N(C_1-C_4 alkyl)(C_1-C_4 alkylphenyl), -NH(C_1-C_4 alkylphenyl), cyano, nitro, oxo (as a substitutent for heteroaryl), -CO_2H, -C(O)OC_1-C_4 alkyl, -CON(C_1-C_4 alkyl)(C_1-C_4 alkyl), -CONH(C_1-C_4 alkyl), -$CONH_2$, -NHC(O)(C_1-C_4 alkyl), -NHC(O)(phenyl), -N(C_1-C_4 alkyl)C(O)(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)C(O)(phenyl), -C(O)C_1-C_4 alkyl, -C(O)C_1-C_4 phenyl, -C(O)C_1-C_4 haloalkyl, -OC(O)C_1-C_4 alkyl, -SO_2(C_1-C_4 alkyl), -SO_2(phenyl), -SO_2(C_1-C_4 haloalkyl), -SO_2NH_2, -SO_2NH(C_1-C_4 alkyl), -SO_2NH(phenyl), -$NHSO_2$(C_1-C_4 alkyl), -$NHSO_2$(phenyl), and -$NHSO_2$(C_1-C_4 haloalkyl).

[0022] **Antimitotic** refers to a drug for inhibiting or preventing mitosis, for example, by causing metaphase arrest. Some antitumour drugs block proliferation and are considered antimitotics.

[0023] **Aryl** and **heteroaryl** mean a 5- or 6-membered aromatic or heteroaromatic ring containing 0 or 1-4 heteroatoms, respectively, chosen from O, N, or S; a bicyclic 9- or 10-membered aromatic or heteroaromatic ring system containing 0 or 1-4 (or more) heteroatoms, respectively, chosen from O, N, or S; or a tricyclic 12- to 14-membered aromatic or heteroaromatic ring system containing 0 or 1-4 (or more) heteroatoms, respectively, chosen from O, N, or S. The aromatic 6- to 14-membered carbocyclic rings include, e.g., phenyl-, naphthyl-, indanyl-, tetralinyl-, and fluorenyl and the 5- to 10-membered aromatic heterocyclic rings include, e.g., imidazolyl-, pyridinyl-, indolyl-, thienyl-, benzopyranonyl-, thiazolyl-, furanyl-, benzimidazolyl-, quinolinyl-, isoquinolinyl-, quinoxalinyl-, pyrimidinyl-, pyrazinyl-, tetrazolyl and pyrazolyl-.

[0024] **Aralkyl-** refers to a residue in which an aryl moiety is attached to the parent structure via an alkyl residue. Examples include benzyl-, phenethyl-, phenylvinyl-, phenylallyl and the like. **Heteroaralkyl-** refers to a residue in which a heteroaryl moiety is attached to the parent structure via an alkyl residue. Examples include furanylmethyl-, pyridinylmethyl-, pyrimidinylethyl and the like.

[0025] **Aralkoxy-** refers to the group -O-aralkyl. Similarly, **heteroaralkoxy-** refers to the group –O-heteroaralkyl-; **aryloxy-** refers to the group –O-aryl-; **acyloxy-** refers to the group –O-acyl-; **heteroaryloxy-** refers to the group –O-heteroaryl-; and **heterocyclyloxy-** refers to the group -O-heterocyclyl (i.e., aralkyl-, heteroaralkyl-, aryl-, acyl-, heterocyclyl-, or heteroaryl is attached to the parent structure through an oxygen).

[0026] **Carboxyalkyl-** refers to the group –alkyl-COOH.

[0027] **Aminocarbonyl** refers to the group -CONR^{b}R^c, where

R^b is H or optionally substituted C_1-C_6 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group; and

R^c is hydrogen or C_1-C_4 alkyl-; and

where each optionally substituted R^b group is independently unsubstituted or substituted with one or more substituents independently chosen from C_1-C_4 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, heteroaryl-C_1-C_4 alkyl-, C_1-C_4 haloalkyl-, -OC_1-C_4 alkyl-, -OC_1-C_4 alkylphenyl, -C_1-C_4 alkyl-OH, -OC_1-C_4 haloalkyl, halogen, -OH, -NH_2, -C_1-C_4 alkyl-NH_2, -N(C_1-C_4 alkyl)(C_1-C_4 alkyl), -NH(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)(C_1-C_4 alkylphenyl), -NH(C_1-C_4 alkylphenyl), cyano, nitro, oxo (as a substitutent for heteroaryl), -CO_2H, -C(O)OC_1-C_4 alkyl, -CON(C_1-C_4 alkyl)(C_1-C_4 alkyl), -CONH(C_1-C_4 alkyl), -CONH_2, -NHC(O)(C_1-C_4 alkyl), -NHC(O)(phenyl), -N(C_1-C_4 alkyl)C(O)(C_1-C_4 alkyl), -N(C_1-C_4 alkyl)C(O)(phenyl), -C(O)C_1-C_4 alkyl, -C(O)C_1-C_4 phenyl, -C(O)C_1-C_4 haloalkyl, -OC(O)C_1-C_4 alkyl, -SO_2(C_1-C_4 alkyl), -SO_2(phenyl), -SO_2(C_1-C_4 haloalkyl), -SO_2NH_2, -SO_2NH(C_1-C_4 alkyl), -SO_2NH(phenyl), -NHSO_2(C_1-C_4 alkyl), -NHSO_2(phenyl), and -NHSO_2(C_1-C_4 haloalkyl). Aminocarbonyl is meant to include carbamoyl-; lower-alkyl carbamoyl-; benzylcarbamoyl-; phenylcarbamoyl-; methoxymethyl-carbamoyl-; and the like.

[0028] **Halogen** or **halo** refers to fluorine, chlorine, bromine or iodine. Fluorine, chlorine and bromine are preferred. Dihaloaryl-, dihaloalkyl-, trihaloaryl etc. refer to aryl and alkyl substituted with the designated plurality of halogens (here, 2, 2 and 3, respectively), but not necessarily a plurality of the same halogen; thus 4-chloro-3-fluorophenyl is within the scope of dihaloaryl-.

[0029] **Heterocyclyl** means a cycloalkyl or aryl residue in which one to four of the carbons is replaced by a heteroatom such as oxygen, nitrogen or sulfur. Examples of heterocycles that fall within the scope of the invention include azetidinyl-, imidazolinyl-, pyrrolidinyl-, pyrazolyl-, pyrrolyl-, indolyl-, quinolinyl-, isoquinolinyl-, tetrahydroisoquinolinyl-, benzofuranyl-, benzodioxanyl-, benzodioxyl (commonly referred to as methylenedioxyphenyl-, when occurring as a substituent), tetrazolyl-, morpholinyl-, thiazolyl-, pyridinyl-, pyridazinyl-, piperidinyl-, pyrimidinyl-, thienyl-, furanyl-, oxazolyl-, oxazolinyl-, isoxazolyl-, dioxanyl-, tetrahydrofuranyl and the like.

"N-heterocyclyl" refers to a nitrogen-containing heterocycle. The term heterocyclyl encompasses heteroaryl-, which is a subset of heterocyclyl-. Examples of N-heterocyclyl residues include azetidinyl-, 4-morpholinyl-, 4-thiomorpholinyl-, 1-piperidinyl-, 1-pyrrolidinyl-, 3-thiazolidinyl-, piperazinyl and 4-(3,4-dihydrobenzoxazinyl). Examples of substituted heterocyclyl include 4-methyl-1-piperazinyl and 4-benzyl-1-piperidinyl-.

[0030] A **leaving group** or **atom** is any group or atom that will, under the reaction conditions, cleave from the starting material, thus promoting reaction at a specified site. Suitable examples of such groups unless otherwise specified are halogen atoms, mesyloxy, p-nitrobenzensulphonyloxy and tosyloxy groups.

[0031] **Optional** or **optionally** means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstances occurs and instances in which it does not. For example, "optionally substituted alkyl" includes "alkyl" and "substituted alkyl" as defined herein. It will be understood by those skilled in the art with respect to any group containing one or more substituents that such groups are not intended to introduce any substitution or substitution patterns that are sterically impractical and/or synthetically non-feasible and/or inherently unstable.

[0032] **Substituted alkoxy** refers to alkoxy wherein the alkyl constituent is substituted (i.e., -O-(substituted alkyl)). One suitable substituted alkoxy group is "polyalkoxy" or -O-(optionally substituted alkylene)-(optionally substituted alkoxy), and includes groups such as $-OCH_2CH_2OCH_3$, and residues of glycol ethers such as polyethyleneglycol, and $-O(CH_2CH_2O)_xCH_3$, where x is an integer of about 2-20, preferably about 2-10, and more preferably about 2-5. Another suitable substituted alkoxy group is hydroxyalkoxy or $-OCH_2(CH_2)_yOH$, where y is an integer of about 1-10, preferably about 1-4.

[0033] **Substituted-** alkyl-, aryl-, and heteroaryl- refer respectively to alkyl-, aryl-, and heteroaryl wherein one or more (in one embodiment, up to about 5; in another embodiment, up to about 3) hydrogen atoms are replaced by a substituent independently chosen from the group: $-R^a$, $-OR^b$, $-O(C_1\text{-}C_2$ alkyl)O- (e.g., ethylenedioxy or methylenedioxy), $-SR^b$, guanidine, guanidine wherein one or more of the guanidine hydrogens are replaced with a lower-alkyl group, $-NR^bR^c$, halogen, cyano, nitro, $-COR^b$, $-CO_2R^b$, $-CONR^bR^c$, $-OCOR^b$, $-OCO_2R^a$, $-OCONR^bR^c$,

$-NR^cCOR^b$, $-NR^cCO_2R^a$, $-NR^cCONR^bR^c$, $-CO_2R^b$, $-CONR^bR^c$, $-NR^cCOR^b$, $-SOR^a$, $-SO_2R^a$, $-SO_2NR^bR^c$, and $-NR^cSO_2R^a$,

where R^a is an optionally substituted C_1-C_6 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group,

R^b is H or optionally substituted C_1-C_6 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, or heteroaryl-C_1-C_4 alkyl- group;

R^c is hydrogen or C_1-C_4 alkyl-;

where each optionally substituted R^a group and R^b group is independently unsubstituted or substituted with one or more substituents independently selected from C_1-C_4 alkyl-, aryl-, heteroaryl-, aryl-C_1-C_4 alkyl-, heteroaryl-C_1-C_4 alkyl-, C_1-C_4 haloalkyl-, $-OC_1$-C_4 alkyl-, $-OC_1$-C_4 alkylphenyl-, $-C_1$-C_4 alkyl-OH, $-OC_1$-C_4 haloalkyl-, halogen, -OH, $-NH_2$, $-C_1$-C_4 alkyl-NH_2, $-N(C_1$-C_4 alkyl$)(C_1$-C_4 alkyl), $-NH(C_1$-C_4 alkyl), $-N(C_1$-C_4 alkyl$)(C_1$-C_4 alkylphenyl), $-NH(C_1$-C_4 alkylphenyl), cyano, nitro, oxo (as a substitutent for heteroaryl), $-CO_2H$, $-C(O)OC_1$-C_4 alkyl-, $-CON(C_1$-C_4 alkyl$)(C_1$-C_4 alkyl), $-CONH(C_1$-C_4 alkyl), $-CONH_2$, $-NHC(O)(C_1$-C_4 alkyl), -NHC(O)(phenyl), $-N(C_1$-C_4 alkyl$)C(O)(C_1$-C_4 alkyl), $-N(C_1$-C_4 alkyl)C(O)(phenyl), $-C(O)C_1$-C_4 alkyl-, $-C(O)C_1$-C_4 phenyl-, $-C(O)C_1$-C_4 haloalkyl-, $-OC(O)C_1$-C_4 alkyl-, $-SO_2(C_1$-C_4 alkyl), $-SO_2$(phenyl), -$SO_2(C_1$-C_4 haloalkyl), $-SO_2NH_2$, $-SO_2NH(C_1$-C_4 alkyl), $-SO_2NH$(phenyl), -$NHSO_2(C_1$-C_4 alkyl), $-NHSO_2$(phenyl), and $-NHSO_2(C_1$-C_4 haloalkyl). In the compounds of Formula I where T and/or T' are substituted lower alkylene, the term "substituted" also refers to alkylene groups where one or more (particularly 1 or 2) carbon atoms are replaced by a heteroatom independently selected from O, N or S, such as $-CH_2$-S-CH_2-.

[0034] **Sulfanyl** refers to the groups: -S-(optionally substituted alkyl), -S-(optionally substituted aryl), -S-(optionally substituted heteroaryl), and -S-(optionally substituted heterocyclyl).

[0035] **Sulfinyl** refers to the groups: -S(O)-H, -S(O)-(optionally substituted alkyl), -S(O)-optionally substituted aryl), -S(O)-(optionally substituted heteroaryl), -S(O)-(optionally substituted heterocyclyl); and -S(O)-(optionally substituted amino).

[0036] **Sulfonyl** refers to the groups: $-S(O_2)$-H, $-S(O_2)$-(optionally substituted alkyl), $-S(O_2)$-(optionally substituted aryl), $-S(O_2)$-(optionally substituted heteroaryl), $-S(O_2)$-(optionally substituted heterocyclyl) ,$-S(O_2)$-(optionally substituted alkoxy),

-$S(O_2)$-(optionally substituted aryloxy), -$S(O_2)$-(optionally substituted heteroaryloxy), -$S(O_2)$-(optionally substituted heterocyclyloxy); and -$S(O_2)$-(optionally substituted amino).

[0037] **Pharmaceutically acceptable salts** refers to those salts that retain the biological utility of the free compound and that are not biologically undesirable or unsuitable for pharmaceutical use, formed with a suitable acid or base, and includes pharmaceutically acceptable acid addition salts and base addition salts.

[0038] **Pharmaceutically acceptable acid addition salts** include those derived from inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and those derived from organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

[0039] **Pharmaceutically acceptable base addition salts** include those derived from inorganic bases such as sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Particular embodiments are the ammonium, potassium, sodium, calcium, and magnesium salts. Base addition salts also include those derived from pharmaceutically acceptable organic non-toxic bases, including salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, and ethanolamine.

[0040] **Protecting group** has the meaning conventionally associated with it in organic synthesis, i.e. a group that selectively blocks one or more reactive sites in a multifunctional compound such that a chemical reaction can be carried out selectively on another unprotected reactive site and such that the group can readily be removed after the selective reaction is complete. A variety of protecting groups are disclosed, for example, in T.H. Greene and P. G. M. Wuts, Protective Groups in Organic Synthesis, Third Edition, John Wiley & Sons, New York (1999), which is incorporated herein by reference in its entirety. For example, a hydroxy protected form is where at least one of the hydroxyl groups present in a compound is protected

with a hydroxy protecting group. Likewise, amines and other reactive groups can similarly be protected.

[0041] **Solvate** refers to the compound formed by the interaction of a solvent and a compound of Formula I or salt thereof. Suitable solvates of the compounds of the Formula I or a salt thereof are pharmaceutically acceptable solvates including hydrates.

[0042] Many of the compounds described herein contain one or more asymmetric centers (e.g. the carbon to which R_2 and $R_{2'}$ are attached where R_2 differs from $R_{2'}$) and can thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that can be defined, in terms of absolute stereochemistry, as (R)- or (S)-. The present invention is meant to include all such possible isomers, including racemic mixtures, optically pure forms and intermediate mixtures. Optically active (R)- and (S)- isomers can be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers. Likewise, all tautomeric forms and rotational isomers are also intended to be included.

[0043] When desired, the R- and S-isomers can be resolved by methods known to those skilled in the art, for example by formation of diastereoisomeric salts or complexes which can be separated, for example, by crystallization; via formation of diastereoisomeric derivatives which can be separated, for example, by crystallization, gas-liquid or liquid chromatography; selective reaction of one enantiomer with an enantiomer-specific reagent, for example enzymatic oxidation or reduction, followed by separation of the modified and unmodified enantiomers; or gas-liquid or liquid chromatography in a chiral environment, for example on a chiral support, such as silica with a bound chiral ligand or in the presence of a chiral solvent. It will be appreciated that where the desired enantiomer is converted into another chemical entity by one of the separation procedures described above, a further step can be required to liberate the desired enantiomeric form. Alternatively, specific enantiomer can be synthesized by asymmetric synthesis using optically active reagents, substrates, catalysts or solvents, or by converting one enantiomer to the other by asymmetric transformation.

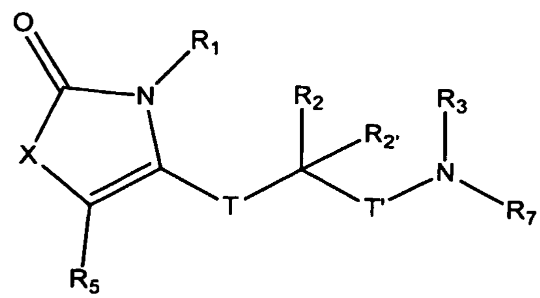
Compounds of the Present Invention

[0044] The present invention is directed to a class of novel compounds that are inhibitors of one or more mitotic kinesins. While not intending to be bound by any theory, the present invention capitalizes on the finding that perturbation of mitotic kinesin function causes malformation or dysfunction of mitotic spindles, frequently resulting in cell cycle arrest and cell death. According to one embodiment of the invention, the compounds described herein inhibit the mitotic kinesin, KSP, and in one embodiment, human KSP. In another embodiment, the compounds inhibit the mitotic kinesin, KSP, as well as modulating one or more of the human mitotic kinesins selected from HSET (see, U.S. Patent No. 6,361,993, which is incorporated herein by reference); MCAK (see, U.S. Patent No. 6,331,424, which is incorporated herein by reference); CENP-E (see, PCT Publication No. WO 99/13061 , which is incorporated herein by reference); Kif4 (see, U.S. Patent No. 6,440,684, which is incorporated herein by reference); MKLP1 (see, U.S. Patent No. 6,448,025, which is incorporated herein by reference); Kif15 (see, U.S. Patent No. 6,355,466, which is incorporated herein by reference); Kid (see, U.S. Patent No. 6,387,644, which is incorporated herein by reference); Mpp1, CMKrp, KinI-3 (see, U.S. Patent No. 6,461,855, which is incorporated herein by reference); Kip3a (see, PCT Publication No. WO 01/96593, which is incorporated herein by reference); Kip3d (see, U.S. Patent No. 6,492,151, which is incorporated herein by reference); and RabK6.

[0045] The methods of inhibiting a mitotic kinesin comprise contacting an inhibitor of the invention with a kinesin, particularly a human kinesin, more particularly, human KSP or fragments and variants thereof. The inhibition can be of the ATP hydrolysis activity of the KSP kinesin and/or the mitotic spindle formation activity, such that the mitotic spindles are disrupted. Meiotic spindles can also be disrupted.

[0046] The present invention provides inhibitors of mitotic kinesins, in particular KSP and especially human KSP, for the treatment of disorders associated with cell proliferation. The compounds, compositions and methods described herein can differ in their selectivity and are used to treat diseases of cellular proliferation, including, but not limited to cancer, hyperplasias, restenosis, cardiac hypertrophy, immune disorders, fungal disorders and inflammation.

[0047] Accordingly, the present invention relates to methods employing compounds represented by Formula I:



Formula I

wherein:

T and T' are independently a covalent bond or optionally substituted lower alkylene;

X is O or $-NR_4$;

R_1 is hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, or optionally substituted heteroaralkyl-;

R_2 and $R_{2'}$ are independently hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, or optionally substituted heteroaralkyl; or R_2 and $R_{2'}$ taken together form an optionally substituted 3- to 7-membered ring which optionally incorporates from one to two heteroatoms, selected from N, O, and S in the ring

R_3 is hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, optionally substituted heteroaralkyl-, $-C(O)-R_6$, or $-S(O)_2-R_{6a}$;

R_4 is hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, or optionally substituted heteroaralkyl-; and R_5 is hydrogen, halogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, or optionally substituted heteroaralkyl-; or R_4 and R_5 taken together with

the carbon and nitrogen to which they are bound, respectively, form an optionally substituted 5- to 7-membered ring;

R_6 is hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, optionally substituted heteroaralkyl, R_9O- or R_{11}-NH-;

R_{6a} is optionally substituted alkyl, optionally substituted aryl, optionally substituted alkylaryl, optionally substituted heteroaryl, optionally substituted alkylheteroaryl, or R_{11}-NH-;

R_7 is hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, or optionally substituted heteroaralkyl;

or R_7 taken together with R_3, and the nitrogen to which they are bound, form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates from one to two additional heteroatoms, chosen from N, O, and S in the heterocycle ring;

or R_7 taken together with R_2 form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates from one to two additional heteroatoms, chosen from N, O, and S in the heterocycle ring;

R_9 is optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, or optionally substituted heteroaralkyl and

R_{11} is hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, or optionally substituted heteroaralkyl;

(Formula I including single stereoisomers and mixtures of stereoisomers);

a pharmaceutically acceptable salt of a compound of Formula I;

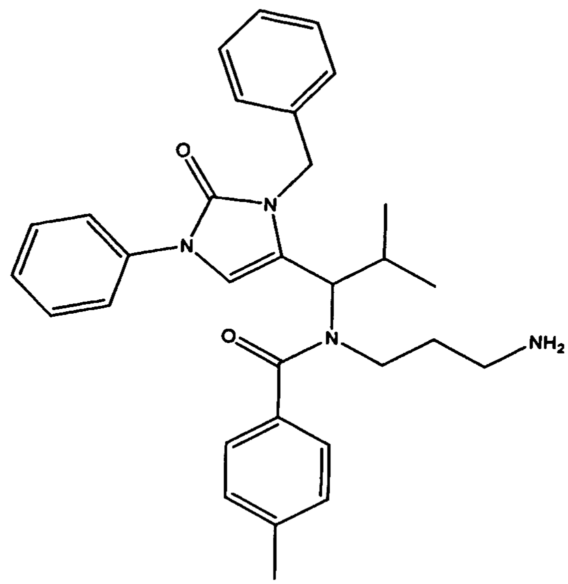
a pharmaceutically acceptable solvate of a compound of Formula I; or

a pharmaceutically acceptable solvate of a pharmaceutically acceptable salt of a compound of Formula I.

[0048] In a particular embodiment, the stereogenic center to which R_2 and $R_{2'}$ are attached is of the R configuration.

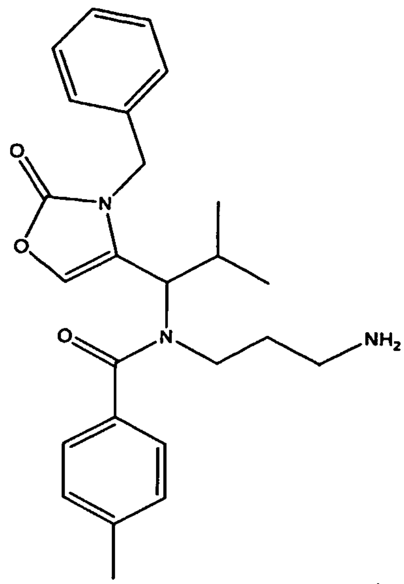
Nomenclature

[0049] The compounds of Formula I can be named and numbered in the manner (e.g., using AutoNom version 2.1 in ChemDraw or ISIS-DRAW) described below.



i.e., the compound according to Formula I where T and T' are a covalent bond; X is –NR_4; R_1 is benzyl-, R_2 is propyl- (or i -propyl), $R_{2'}$ is hydrogen; R_3 is COR_6; R_4 is phenyl-; R_5 is hydrogen; R_7 is 3-aminopropyl-; and R_6 is p-tolyl- can be named N-(3-amino-propyl)-N-[1-(3-benzyl-2-oxo-1-phenyl-2,3-dihydro-1H-imidazol-4-yl)-2-methyl-propyl]-4-methyl-benzamide.

[0050] Likewise, the compound having the structure



i.e., the compound according to Formula I where T and T' are a covalent bond; X is O; R_1 is benzyl-, R_2 is propyl- (or i –propyl-), $R_{2'}$ is hydrogen; R_3 is $-COR_6$; R_5 is hydrogen; R_7 is 3-aminopropyl-; and R_6 is p-tolyl- can be named N-(3-amino-propyl)-N-[1-(3-benzyl-2-oxo-2,3-dihydro-oxazol-4-yl)-2-methyl-propyl]-4-methyl-benzamide.

Synthetic Reaction Parameters

[0051] The compounds of Formula I can be prepared by following the procedures described with reference to the Reaction Schemes below.

[0052] The optionally substituted compounds of Formula 101 and other reactants are commercially available, e.g., from Aldrich Chemical Company, Milwaukee, WI, or can be readily prepared by those skilled in the art using commonly employed synthetic methodology.

[0053] Unless specified otherwise, the terms "solvent", "inert organic solvent" or "inert solvent" mean a solvent inert under the conditions of the reaction being described in conjunction therewith [including, for example, benzene, toluene, acetonitrile, tetrahydrofuran ("THF"), dimethylformamide ("DMF"), chloroform, methylene chloride (or dichloromethane), diethyl ether, methanol, pyridine and the like]. Unless specified to the contrary, the solvents used in the reactions of the present

invention are inert organic solvents.

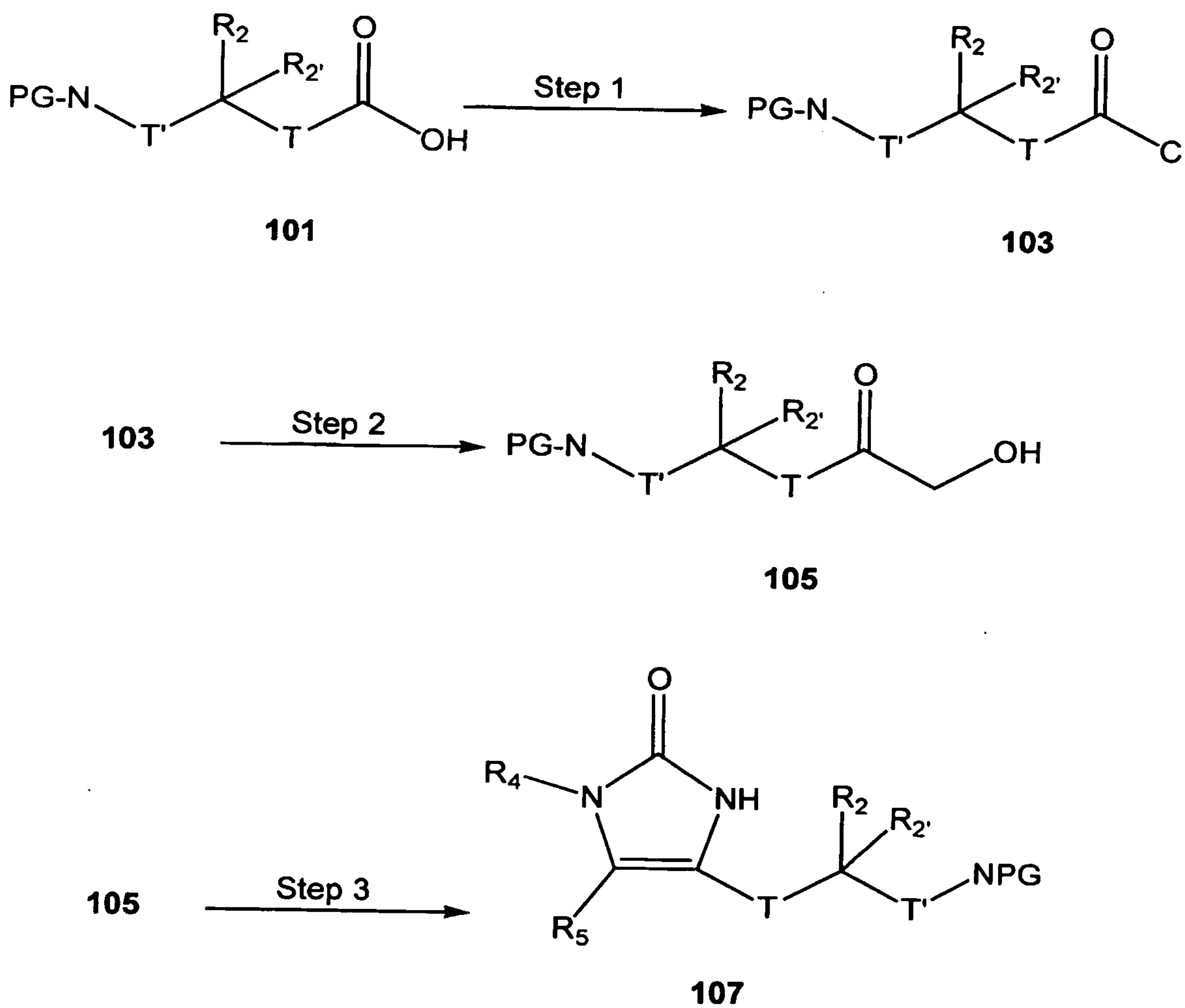
[0054] In general, esters of carboxylic acids can be prepared by conventional esterification procedures, for example alkyl esters can be prepared by treating the required carboxylic acid with the appropriate alkanol, generally under acidic conditions. Likewise, amides can be prepared using conventional amidation procedures, for example amides can be prepared by treating an activated carboxylic acid with the appropriate amine. Alternatively, a lower-alkyl ester such as a methyl ester of the acid can be treated with an amine to provide the required amide, optionally in presence of trimethylalluminium following the procedure described in Tetrahedron Lett. 48, 4171-4173, (1977). Carboxyl groups can be protected as alkyl esters, for example methyl esters, which esters can be prepared and removed using conventional procedures, one convenient method for converting carbomethoxy to carboxyl is to use aqueous lithium hydroxide.

[0055] The salts and solvates of the compounds mentioned herein can as required be produced by methods conventional in the art. For example, if an inventive compound is an acid, a desired base addition salt can be prepared by treatment of the free acid with an inorganic or organic base, such as an amine (primary, secondary, or tertiary); an alkali metal or alkaline earth metal hydroxide; or the like. Illustrative examples of suitable salts include organic salts derived from amino acids such as glycine and arginine; ammonia; primary, secondary, and tertiary amines; such as ethylenediamine, and cyclic amines, such as cyclohexylamine, piperidine, morpholine, and piperazine; as well as inorganic salts derived from sodium, calcium, potassium, magnesium, manganese, iron, copper, zinc, aluminum, and lithium.

[0056] If a compound is a base, a desired acid addition salt can be prepared by any suitable method known in the art, including treatment of the free base with an inorganic acid, such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like, or with an organic acid, such as acetic acid, maleic acid, succinic acid, mandelic acid, fumaric acid, malonic acid, pyruvic acid, oxalic acid, glycolic acid, salicylic acid, pyranosidyl acid, such as glucuronic acid or galacturonic acid, alpha-hydroxy acid, such as citric acid or tartaric acid, amino acid, such as aspartic acid or glutamic acid, aromatic acid, such as benzoic acid or cinnamic acid, sulfonic acid, such as p-toluenesulfonic acid, methanesulfonic acid, ethanesulfonic acid, or the like.

[0057] Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography or thick-layer chromatography, or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by reference to the examples hereinbelow. However, other equivalent separation or isolation procedures can, of course, also be used.

Reaction Scheme 1



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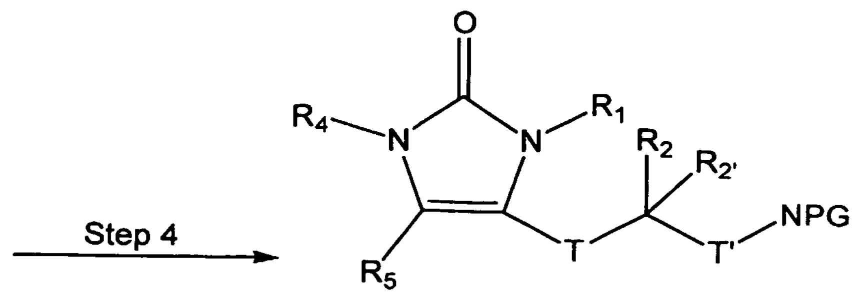


109

Preparation of Compounds of Formula 103

[0058] Refering to Reaction Scheme 1, Step 1, a suspension of a compound of Formula 101, preferably wherein the amine is protected as the phthalimide and an excess (preferably about 1.1 equivalents) of phosphorus pentachloride in an anhydrous, aprotic, nonpolar solvent such as benzene is heated to about 55 °C for about one hour. The product, a compound of Formula 103, is isolated and used in the next step without purification.

Preparation of Compounds of Formula 105

[0059] Refering to Reaction Scheme 1, Step 2, a mixture of a compound of Formula 103 and an excess (preferably about two equivalents) of 1,1,2-tris(trimethylsilyloxy)ethylene is stirred at about 100 °C for about 4 hours. The resulting solution is cooled to room temperature and treated with a solution of aqueous hydrochloric acid in dioxane. The resulting mixture is then heated to about 85 °C for about 30 minutes and cooled to room temperature. The product, a compound of Formula 105, is isolated and used without purification.

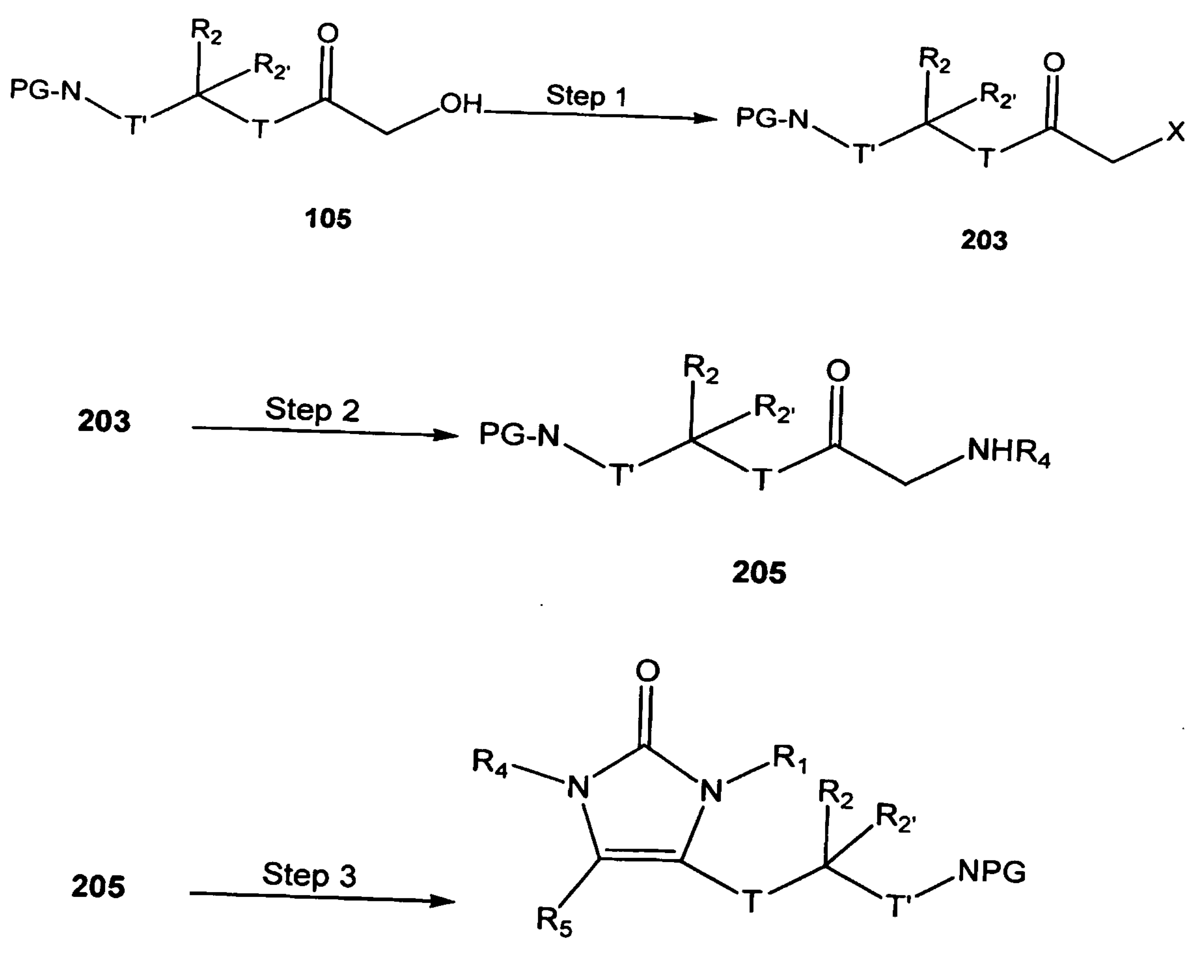
Preparation of Compounds of Formula 107

[0060] Refering to Reaction Scheme 1, Step 3, to a room temperature solution of a compound of Formula 105 and an excess (preferably about 1.5 equivalents) of a compound of the formula R_4-$HNCONH_2$ in a nonpolar, aprotic solvent such as toluene is added trifluoroacetic acid. The resulting solution is sealed and stirred at about 110 °C for about 20 hours. The product, a compound of Formula 107, is isolated and purified.

Preparation of Compounds of Formula 109

[0061] Refering to Reaction Scheme 1, Step 4, to a room temperature solution of a compound of Formula 107 in a polar, aprotic solvent such as dioxane are added successively lithium hydride and an excess (preferably about 1.75 equivalents) of a compound of the formula R_1-X wherein X is a leaving group, preferably tosylate. The resulting solution is heated to about 60 °C for about 24 hours. The product, a compound of Formula 109, is isolated and purified.

Reaction Scheme 2



105 203 205 109

Preparation of Compounds of Formula 203

[0062] Refering to Reaction Scheme 2, Step 1, a hydroxyl group of a compound of Formula 105 is converted to a leaving group, X. In one embodiment,

the leaving group is a mesyl group (although compounds with other leaving groups could be readily prepared using methods known to those skilled in the art.) To a solution of a compound of Formula 105 and a base such as diisopropylethylamine in a nonpolar, aprotic solvent such as dichloromethane at about 0 °C is added a solution of an excess (preferably about 1.1 equivalents) of methanesulfonyl chloride in a nonpolar, aprotic solvent such as dichloromethane. The resulting solution is stirred at about the same temperature for about one hour. The product, a compound of Formula 203 wherein X is -OMs, is isolated and purified.

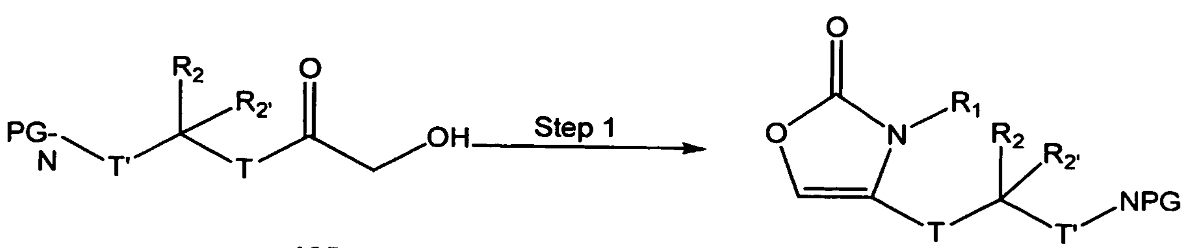
Preparation of Compounds of Formula 205

[0063] Refering to Reaction Scheme 2, Step 2, to a room temperature solution of a compound of Formula 203 in a polar, aprotic solvent such as N,N-dimethylformamide is added an excess (preferably about 1.2 equivalents) of a compound of formula R_4NH_2. The resulting solution is stirred at about 100 °C for about 20 hours. The product, a compound of Formula 205, is isolated and purified.

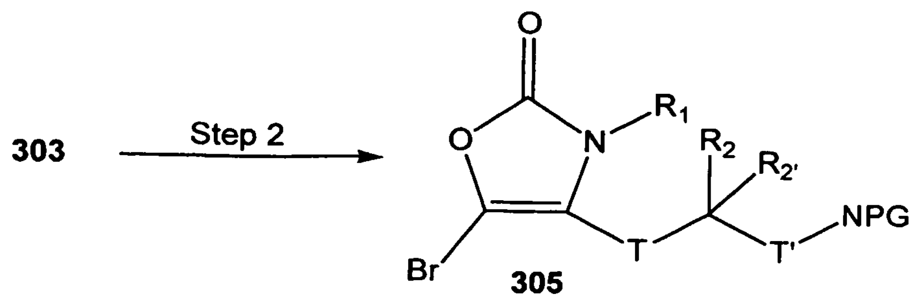
Preparation of Compounds of Formuls 109

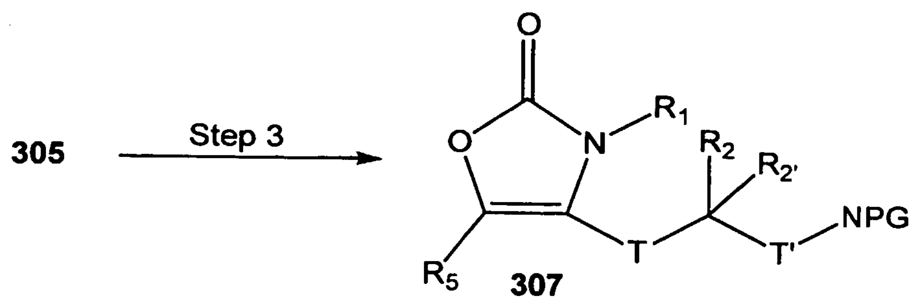
[0064] Refering to Reaction Scheme 2, Step 2, to a room temperature solution of a compound of Formula 205 in a nonpolar, aprotic solvent such as toluene is added an excess (preferably about 2.5 equivalents) of a compound of Formula R_1NCO. The resulting solution is stirred at about 110 °C for about 20 hours and cooled to room temperature. The product, a compound of Formula 109, is isolated and purified.

Reaction Scheme 3



105 303





Preparation of Compounds of Formula 303

[0065] Referring to Reaction Scheme 3, Step 1, to a room temperature solution of a compound of Formula 105 in a polar, aprotic solvent such as N,N-dimethylformamide is added an excess (preferably about 1.4 equivalents) of a compound of formula R_1NCO. The resulting solution is stirred at about 100 °C for about 2 hours under nitrogen and then cooled to room temperature. The desired intermediate is isolated and purified.

[0066] A solution of the intermediate above in glacial acetic acid is refluxed for about 8 hours and cooled to room temperature. The product, a compound of Formula 303, is isolated and used in the next step without purification.

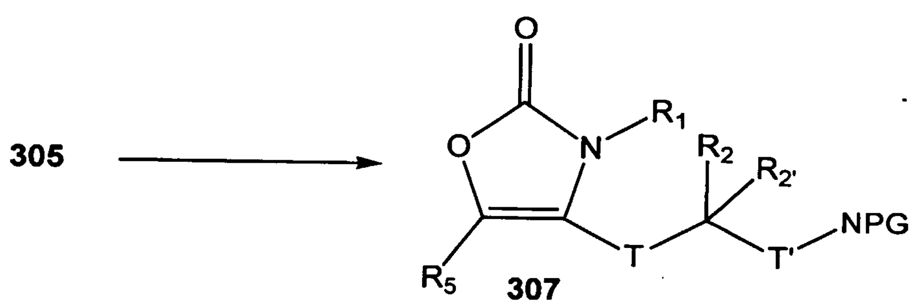
Preparation of Compounds of Formula 305

[0067] Referring to Reaction Scheme 3, Step 2, to a room temperature solution of a compound of Formula 303 in chloroform is added an excess of bromine. The evolved hydrogen bromide is continually displaced by a free-flowing stream of nitrogen, and the resulting solution is stirred for about 1 hour at the same temperature. The product, a compound of Formula 305, is isolated and purified.

Preparation of Compounds of Formula 307

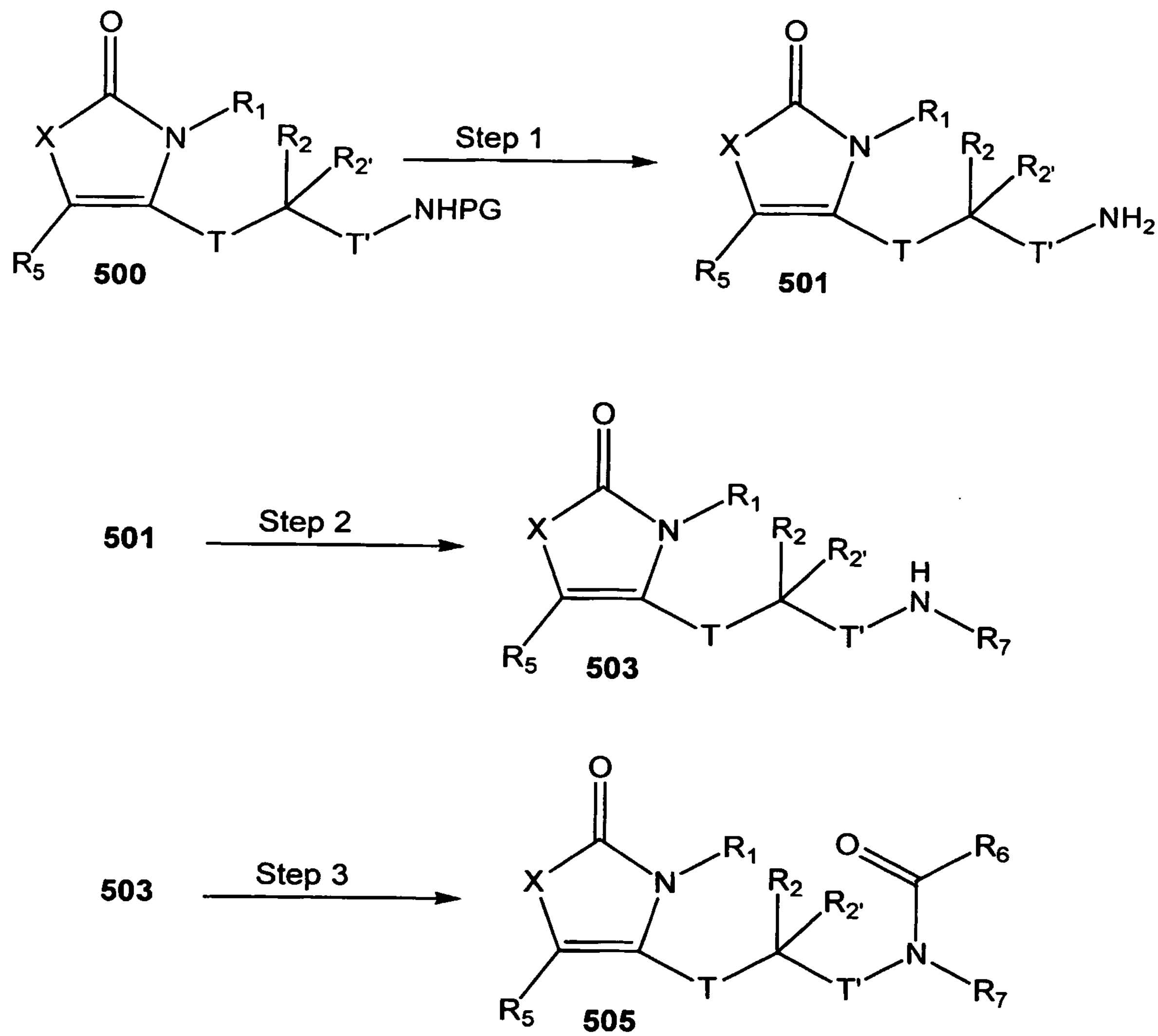
[0068] Referring to Reaction Scheme 3, Step 3, to a compound of Formula 305 is added an excess (preferably about 1.5 equivalents) of a compound of Formula $R_5B(OH)_2$ (preferably, wherein R_5 is phenyl-); palladium(II) acetate (about 1 mol %); 2-(dicyclohexyl)phosphinobiphenyl (about 2 mol %); and an excess (preferably about three equivalents) of potassium fluoride. (One of skill in the art will appreciate that this reaction may also be accomplished with other catalysts and bases.) The flask is flushed by nitrogen three times. A nonpolar, aprotic solvent such as toluene is added, and the resulting mixture is then stirred at about 110 °C for about 48 hours and cooled to room temperature. The product, a compound of Formula 307, is isolated and purified.

Reaction Scheme 4



[0069] Referring to Reaction Scheme 4, to a thick-walled glass tube containing a compound of Formula 305; tri-*o*-tolylphosphine (preferably about 8 mol %), an excess (preferably about two equivalents) of a compound of the formula $(R_5)_4Sn$ (preferably tetramethyltin); and palladium(II) acetate (preferably about 2 mol %) are added *N, N*-dimethylformamide and a base such as triethylamine. (One of skill in the art will appreciate that this reaction may also be accomplished with other catalysts and tin reagents.) The resulting solution is purged with nitrogen, and the tube is quickly sealed and heated to about 115 °C for about 60 hours. It is then cooled to room temperature. The product, a compound of Formula 307, is isolated and purified.

Reaction Scheme 5



Preparation of Compounds of Formula 501

[0070] Referring to Reaction Scheme 5, Step 1, the amino protecting group is removed. When the amino protecting group is phthalimide, this can be accomplished by treating a room temperature solution of a compound of Formula 500 in a polar, protic solvent such as ethanol with a solution of hydrazine in a polar, aprotic solvent such as tetrahydrofuran (preferably as a 1 M solution). The resulting solution is stirred at about 55 °C for about 20 hours and then cooled to room temperature. The product, a

compound of Formula 501, is isolated and purified.

Preparation of Compounds of Formula 503

[0071] Referring to Reaction Scheme 5, Step 2, to a room temperature solution of a compound of Formula 501 in a nonpolar, aprotic solvent such as dichloromethane are added successively an excess (preferably about 1.2 equivalents) of sodium triacetoxyborohydride and an excess (preferably about 1.3 equivalents) of an aldehyde comprising $R_{7'}$ (i.e., a compound having the formula $R_{7'}CHO$ where $R_{7'}CH_2$- is equivalent to R_7 and R_7 is as described above or is a protected precursor to such a substituent, e.g., (3-oxo-propyl)-carbamic acid *tert*-butyl ester). The resulting mixture is stirred at the same temperature under nitrogen for about 12 hours. The product, a compound of Formula 503, is isolated and purified.

Preparation of Compounds of Formula 505

[0072] Refering to Reaction Scheme 5, Step 3, to a solution of a compound of Formula 503 in a nonpolar, aprotic solvent such as dichloromethane at about 0°C are added a base such as DIEA and an excess (preferably about 1.1 equivalents) of an acid chloride of Formula R_6-(CO)-Cl. The resulting solution is stirred under nitrogen at room temperature overnight. The product, a compound of Formula 505, is isolated and purified.

[0073] In an embodiment wherein R_7 further comprises a protected amine, the protecting group may be removed. For example, when the amino protecting group is Boc, this may be accomplished by treating a solution of a compound of Formula 505 in a nonpolar, aprotic solvent such as CH_2Cl_2 with trifluoroacetic acid. The product, the corresponding free amine, is isolated and purified.

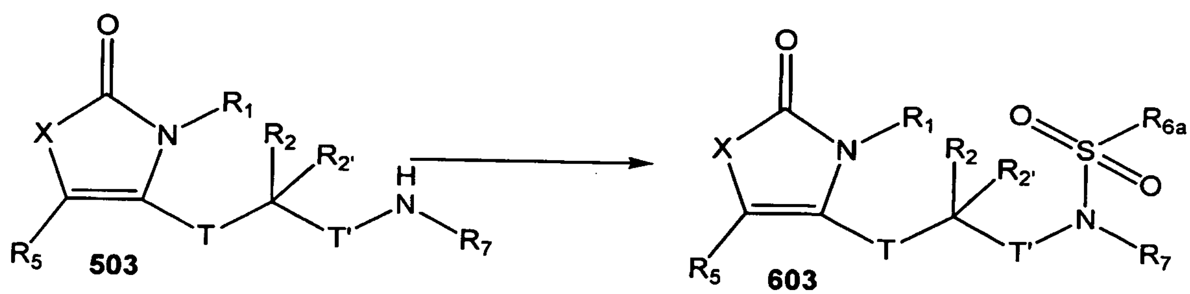
Preparation of Optically Active Compounds

[0074] In certain compounds of the invention, a particular stereo configuration (such as the (R) isomer) may be preferred at the stereogenic center to which R_2 is attached. In certain embodiments, optically active compounds are prepared from optiocally active starting materials. In other embodiments, the optically active compound can be prepared by methods known in the art. For example, an amine of Formula 501 is dissolved in an inert organic solvent (such as IPA) and warmed to

60°C. In a separate vessel, a resolving agent (such as dibenzoyl-D-tartaric acid) is dissolved, preferably in the same warm solvent, and then quickly added (with agitation) to the warm amine solution. The reaction mixture is left to crystallize by cooling to room temperature over 16 hours under continuing agitation. The desired isomer is isolated and purified. In certain embodiments, the desired isomer is isolated by chiral chromatography.

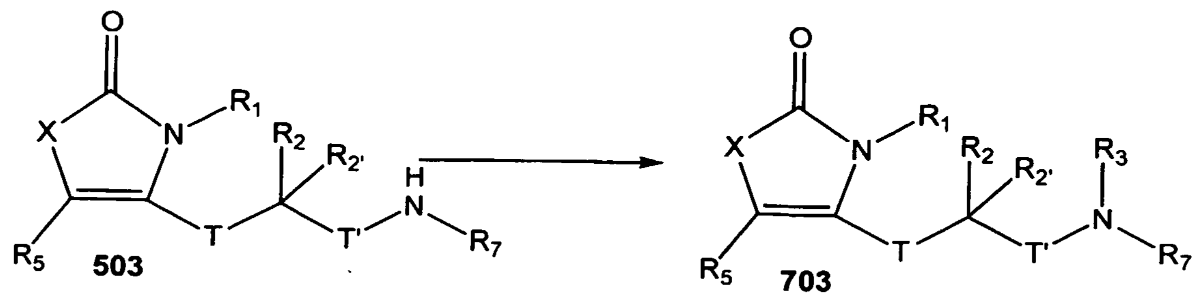
[0075] For the sake of brevity in the remaining description of the synthesis of compounds of Formula I, it should be understood that either single isomer or a mixture of isomers may be employed to give the corresponding product.

Reaction Scheme 6



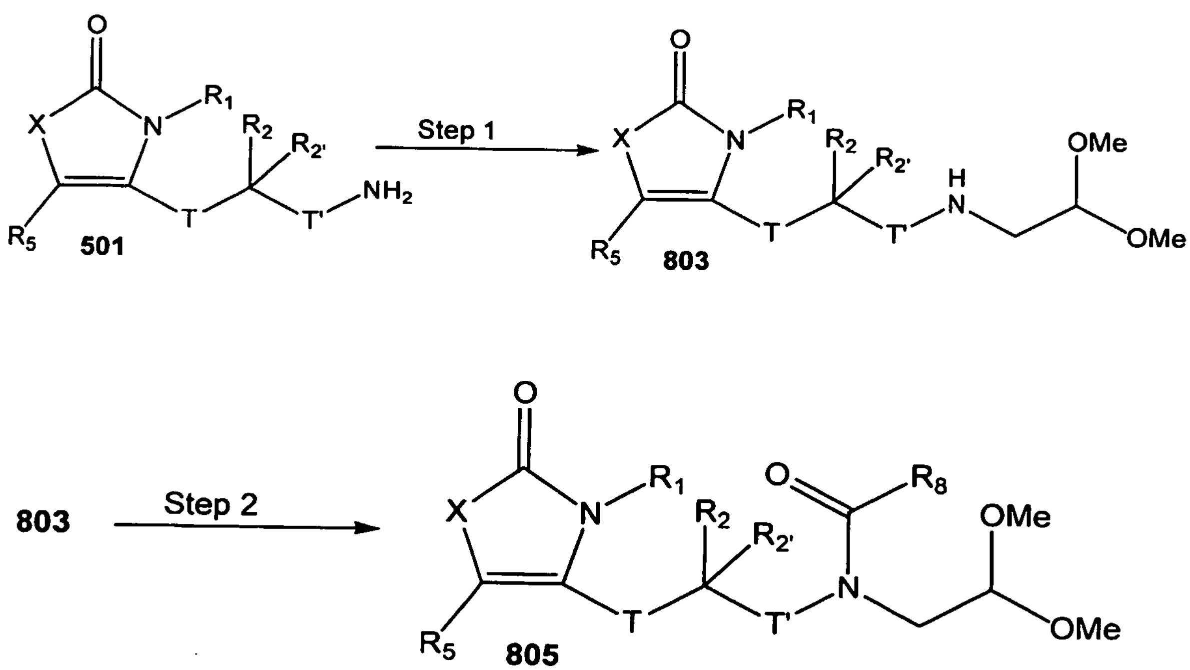
[0076] Referring to Reaction Scheme 6, to a solution of a compound of Formula 503 and an amine base such as diisopropylethylamine in a nonpolar, aprotic solvent such as dichloromethane is added a compound having the formula $Cl\text{-}S(O)_2\text{-}R_{6a}$ or $O\text{-}(S(O)_2\text{-}R_{6a})_2$ where R_{6a} is as described above. The resulting solution is stirred under nitrogen at room temperature for several hours. The product, a compound of Formula 603, is isolated and purified.

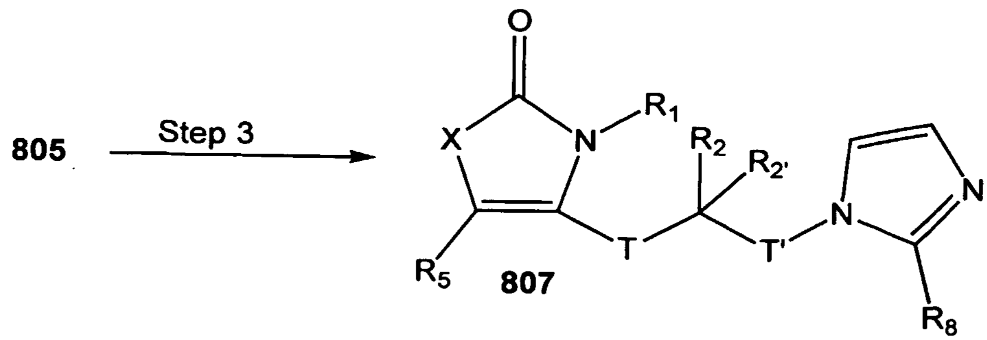
Reaction Scheme 7



[0077] Referring to Reaction Scheme 7, to a solution of a compound of Formula 503 and an amine base such as diisopropylethylamine in a nonpolar, aprotic solvent such as dichloromethane is added a compound having the formula X- R_3 where R_3 is as described above and X is a leaving group (such as halogen or tosylate). The resulting solution is stirred under nitrogen at room temperature or with heat for several hours. The product, a compound of Formula 703, is isolated and purified.

Reaction Scheme 8





Preparation of Formula 803

[0078] Referring to Reaction Scheme 8, Step 1, to an optionally substituted compound of Formula 501 dissolved in a polar, aprotic solvent (such as DMF) in the presence of a base (such as potassium carbonate) is added one equivalent of an optionally substituted suitably protected aldehyde wherein such aldehyde further comprises a leaving group, preferably, a halide (such as bromoacetaldehyde dimethylacetal). The solution is heated at reflux, monitoring completion of the reaction (e.g., by TLC). The reaction mixture is cooled and the corresponding, optionally substituted compound of Formula 803 is isolated and purified.

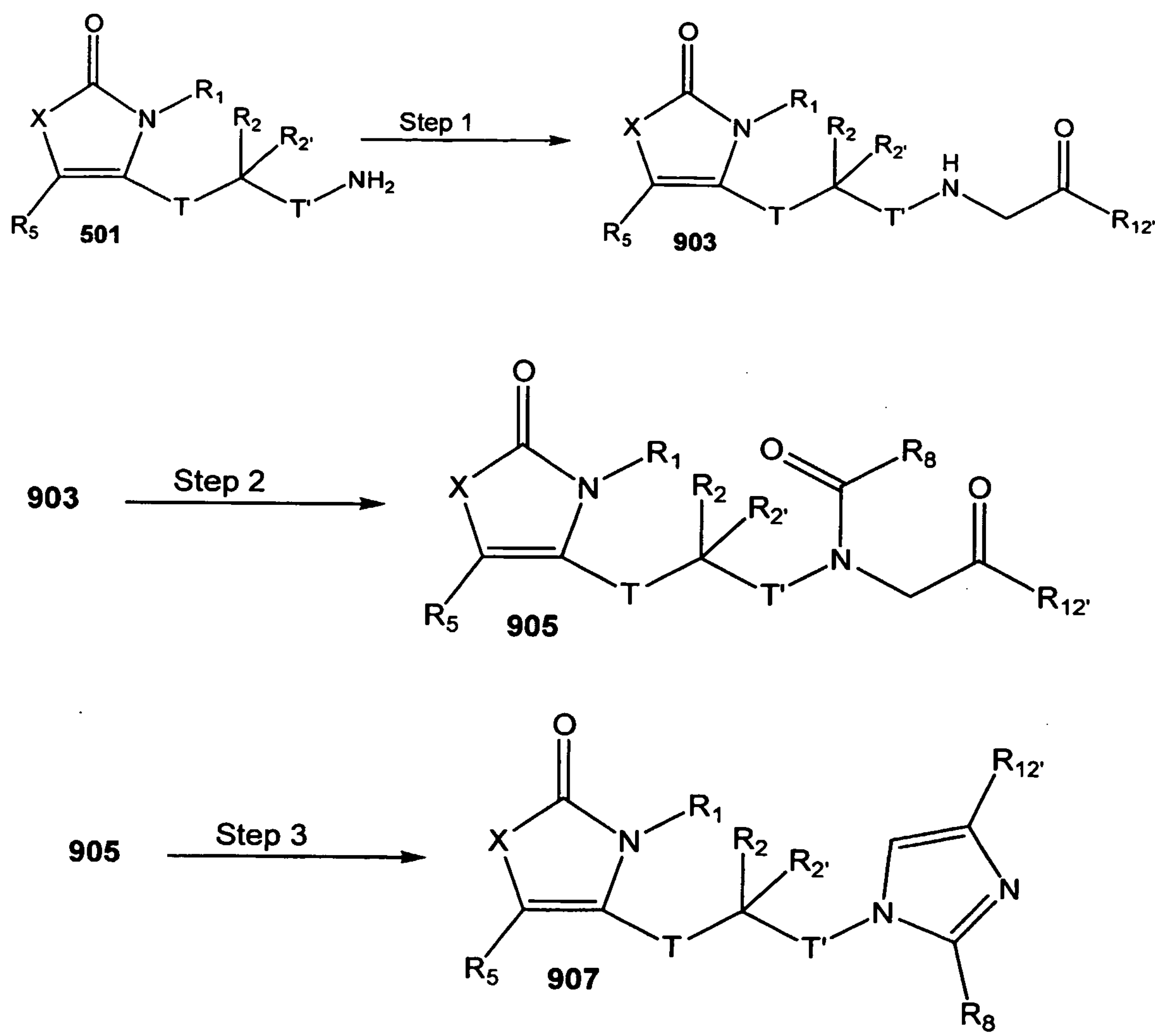
Preparation of Formula 805

[0079] Referring to Reaction Scheme 8, Step 2, to an optionally substituted compound of Formula 803 in an inert solvent (such as dichloromethane) in the presence of about 1.5 molar equivalents of an amine base (such as triethylamine) is added about 1.5 molar equivalents of an R_8 acid chloride, such as, Cl-C(O)-R_8, where R_8 is as described herein. The reaction takes place, with stirring, at room temperature over a period of 4 to 24 hours. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 805 is isolated and purified.

Preparation of Formula 807

[0080] Referring to Reaction Scheme 8, Step 3, a solution of a compound of Formula 805 and an excess of ammonium acetate in acetic acid is heated at reflux for 1-4 hours. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 807 is isolated and purified.

Reaction Scheme 9



Preparation of Formula 903

[0081] Referring to Reaction Scheme 9, Step 1, a suspension of a compound of Formula 501, an alpha-haloketone reagent of the Formula $R_{12'}(CO)CH_2Y$ wherein Y is a leaving group (preferably, a halide) and $R_{12'}$ is as described herein, and about an equivalent of a base, such as potassium carbonate in a polar, aprotic solvent such as DMF is stirred at room temperature. The reaction is diluted with water and the resulting solid, a compound of Formula 903, is used in the subsequent step without further purification.

Preparation of Formula 905

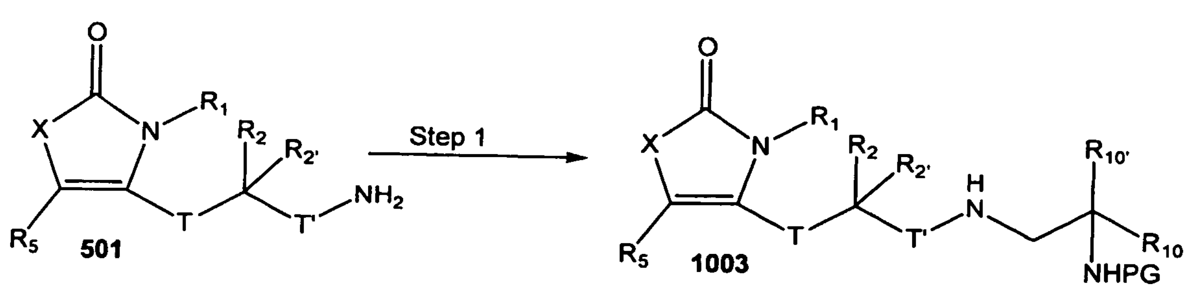
[0082] Referring to Reaction Scheme 9, Step 2, a solution of the compound of Formula 903, about an equivalent of an amine base, such as triethylamine and about an equivalent of an acid chloride (such as a compound of Formula R_8-COCl) in an organic solvent such as methylene chloride is stirred at room temperature for several hours. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 905 is isolated and purified.

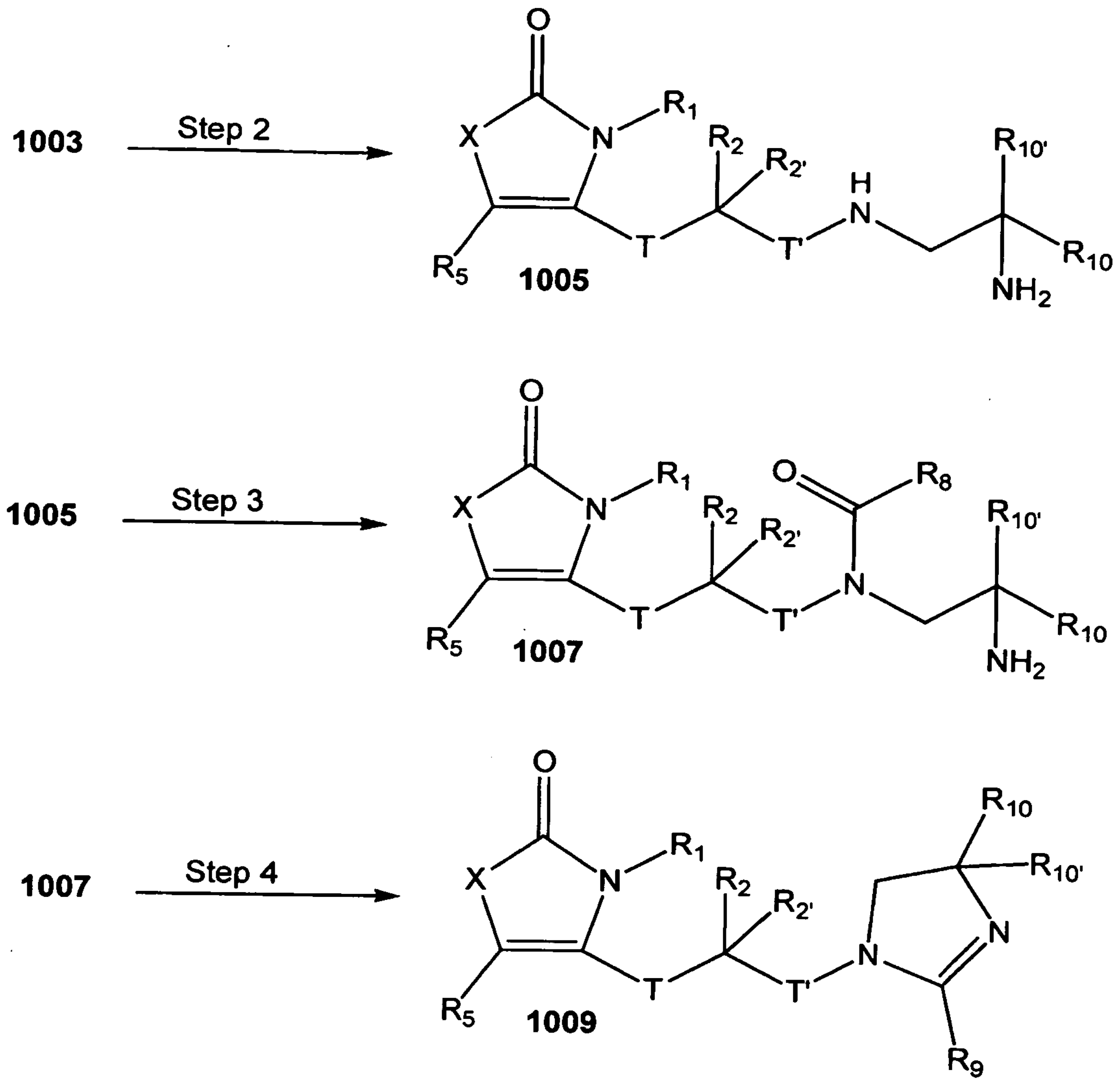
Preparation of Formula 907

[0083] Referring to Reaction Scheme 9, Step 3, a solution of a compound of Formula 905 and an excess of ammonium acetate in acetic acid is heated at reflux using a Dean-Stark trap and condenser. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 907 is isolated and purified.

[0084] In an embodiment when $R_{12'}$ comprises a protected aminoalkyl group, the amino protected group may be removed. For example, when the amino group is protected as the corresponding isoindole-1,3-dione, a solution of a compound of Formula 507 and an excess of anhydrous hydrazine in a polar, protic solvent such as ethanol is heated at reflux. The reaction is cooled to about 5^{o}C and any precipitate is filtered off. The filtrate is concentrated in vacuo and purified to yield the corresponding free amine.

Reaction Scheme 10





Preparation of Formula 1003

[0085] Referring to Reaction Scheme 10, Step 1, reductive amination of amines of Formula 501 with an optionally substituted, aldehyde-containing carbamic acid ester gives urethane intermediates. More specifically, to a solution of a compound of Formula 501 and an equivalent of a suitably protected aldehyde (Seki *et. al. Chem. Pharm. Bull.* 1996, *44*, 2061) in dichloromethane is added a slight excess of a reducing agent, such as sodium triacetoxyborohydride. The resultant cloudy mixture is maintained at ambient temperature. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 1003 is isolated and used in the subsequent step without purification.

Preparation of Formula 1005

[0086] Referring to Reaction Scheme 10, Step 2, to a solution of a compound of Formula 1003 in a polar, aprotic solvent such as dichloromethane is added a strong acid such as trifluoroacetic acid. The resultant solution is maintained at ambient temperature overnight and concentrated under reduced pressure. The residue is isolated to give a compound of Formula 1005 which was used in the subsequent step without purification.

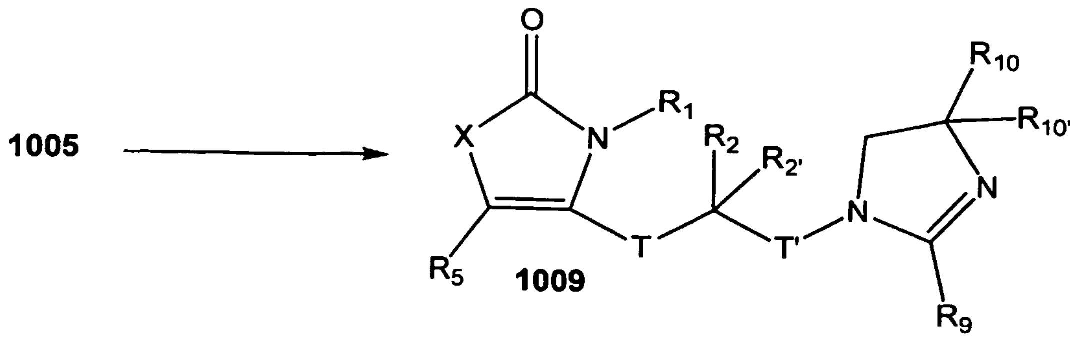
Preparation of Formula 1007

[0087] Referring to Reaction Scheme 10, Step 3, to a solution of a compound of Formula 1005 in a polar, aprotic solvent such as dichloromethane is added an excess, preferably about two equivalents of an amine base such as triethylamine, followed by about an equivalent or slight excess of an acid chloride. The resultant solution is stirred at ambient temperature for about 3 hours. Completion is monitored, e.g., by TLC. The corresponding compound of Formula 1007 is isolated and purified.

Preparation of Formula 1009

[0088] Referring to Reaction Scheme 10, Step 4, a solution of a compound of Formula 1007 in an excess of phosphorus oxychloride is heated at reflux. After 8 hours, the reaction mixture is allowed to cool to ambient temperature and concentrated under reduced pressure. The corresponding compound of Formula 1009 is isolated and purified.

Reaction Scheme 11

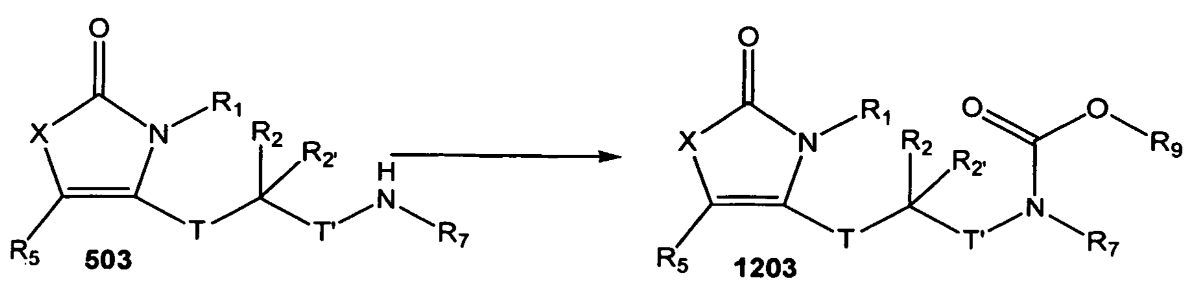


Preparation of Formula 1009

[0089] As an alternative to Steps 3 and 4 of Reaction Scheme 10, acylation of primary amines of Formula 1005, followed by acetic acid mediated cyclization, can proceed without isolation of the intermediate amides to provide the target compound of Formula 1009. This route is shown in Reaction Scheme 11.

[0090] More specifically, to a solution of a compound of Formula 1005 in a nonpolar, aprotic solvent such as dichloromethane is added an excess, preferably about two equivalents of an amine base, such as triethylamine, followed by about an equivalent of an acid chloride. The resultant solution is stirred at ambient temperature for 2 hours, then evaporated under reduced pressure. The resultant solid is treated with glacial acetic acid, then the resultant suspension is heated at reflux for about 48 hours. The reaction is cooled to ambient temperature then evaporated under reduced pressure. The corresponding compound of Formula 1009 is isolated and purified.

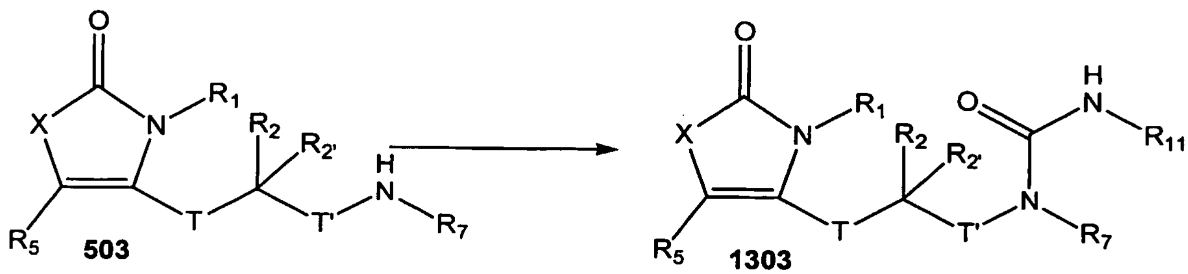
Reaction Scheme 12



[0091] Referring to Reaction Scheme 12, a compound of Formula 503 is reacted with a slight excess of a compound of the formula $R_9O(CO)Cl$ in the presence

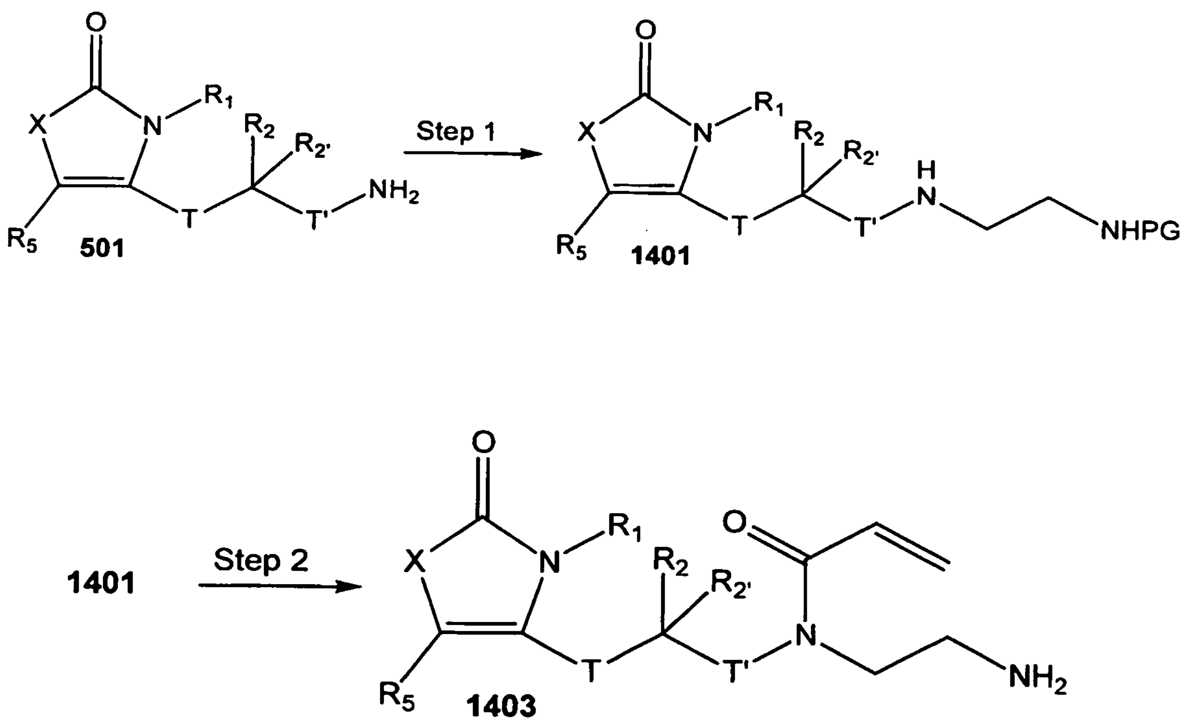
of a base such as triethylamine in a nonpolar, aprotic solvent such as dichloromethane. The product, a compound of Formula 1203 is isolated and purified.

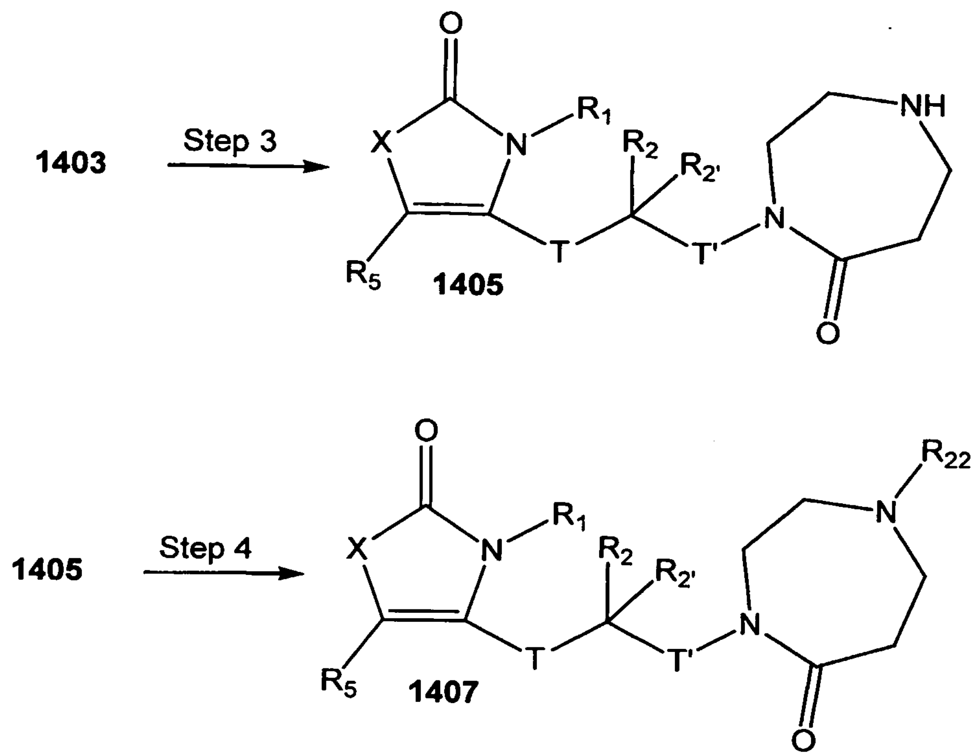
Reaction Scheme 13



[0092] Referring to Reaction Scheme 13, a compound of Formula 503 is treated with a slight excess of an isocyanate R_{11}-N=C=O in the presence of a base, such as triethylamine, in a nonpolar, aprotic solvent, such as dichloromethane. The product, a compound of Formula 1303, is isolated and purified.

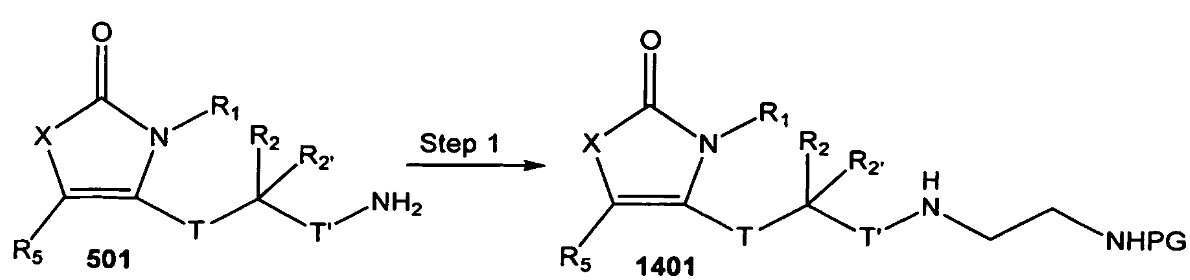
Reaction Scheme 14

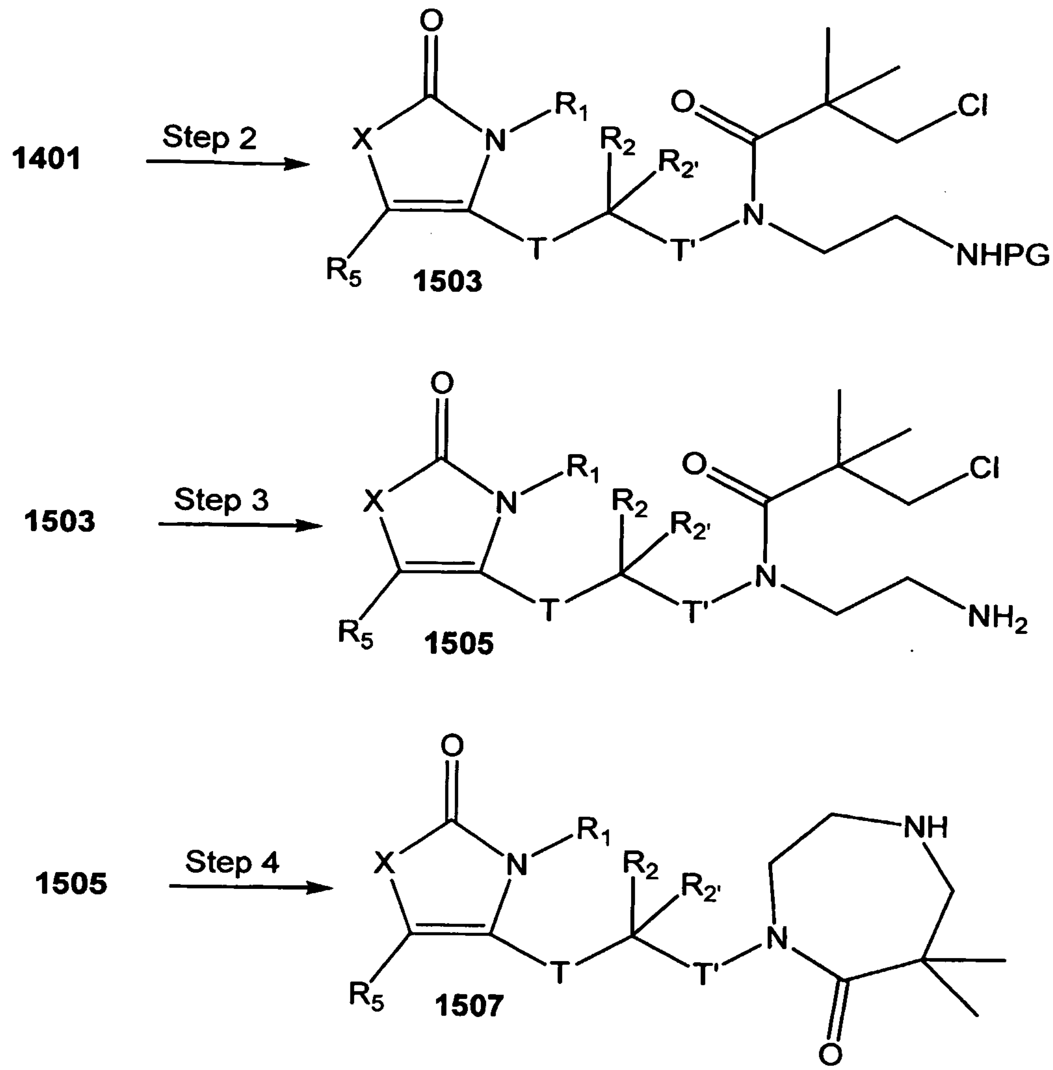




[0093] Referring to Reaction Scheme 14, reductive amination of the primary amino group in compounds of Formula 501 with (2-oxo-ethyl)-carbamic acid *tert*-butyl ester gives the corresponding secondary amine. Acylation with acryloyl chloride followed by deprotection of the tertiary amide and base mediated cyclisation gives the desired diazepanones. If desired, further functionalization of the basic amine can be accomplished under conditions well known to those skilled in the art.

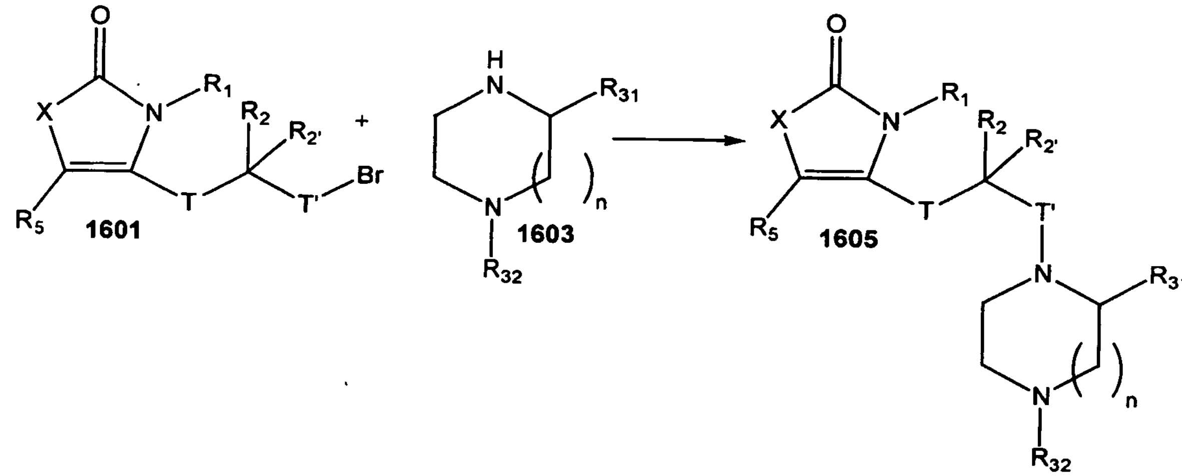
Reaction Scheme 15





[0094] Referring to Reaction Scheme 15, reductive amination of the primary amino group in compounds of Formula 501 with (2-oxo-ethyl)-carbamic acid *tert*-butyl ester gives the corresponding secondary amine. Acylation with chloropivaloyl chloride followed by deprotection of the tertiary amide and base mediated cyclisation gives the desired diazepanones. If desired, further functionalization of the basic amine can be accomplished under conditions well known to those skilled in the art.

Reaction Scheme 16



[0095] Referring to Reaction Scheme 16, a compound of Formula 1601, one-half molar equivalent of an optionally substituted piperazine or diazepam (as shown above, where R_{32} is as described herein) and an excess of potassium carbonate are combined in an organic solvent (e.g., acetonitrile). The reaction takes place under a nitrogen atmosphere at elevated temperature (e.g., 100°C) over a period of 8 hours, followed at a somewhat lower temperature (e.g., 60°C) for a period of 5 days. The product, a compound of Formula 1603, is isolated and purified.

[0096] Optionally, in the event that R_{32} is an amine protecting group, such as Boc, it can be removed by for example treatment with a 95/5 mixture of TFA/water followed by stirring at room temperature for 1 hour. The product, a compound of Formula 1603 wherein R_{32} is hydrogen, can be isolated and purified. If desired, further functionalization of the basic amine could be accomplished under conditions well known to those skilled in the art.

Particular Processes

[0097] A compound of Formula I is optionally contacted with a pharmaceutically acceptable acid or base to form the corresponding acid or base addition salt.

[0098] A pharmaceutically acceptable acid addition salt of a compound of Formula I is optionally contacted with a base to form the corresponding free base of

Formula I. A pharmaceutically acceptable base addition salt of a compound of Formula I is optionally contacted with an acid to form the corresponding free acid of Formula I.

[0099] A racemic mixture of isomers of a compound of Formula I is placed on a chromatography column and separated into (R)- and (S)- enantiomers.

Compounds

T and T'

[00100] When considering the compounds of the invention, T is optionally substituted lower alkylene or is covalent bond; and T' is optionally substituted lower alkylene or is a covalent bond. In one embodiment, one of T and T' is a covalent bond and the other is optionally substituted lower alkylene (especially optionally substituted methylene). In another embodiment, both are covalent bonds.

R_1

[00101] When considering the compounds of the invention, in a particular embodiment R_1 is hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aralkyl, or optionally substituted heteroaralkyl. In a more particular embodiment R_1 is optionally substituted lower alkyl, optionally substituted aryl, or optionally substituted aralkyl (especially optionally substituted aralkyl).

[00102] In a most particular embodiment R_1 is ethyl, propyl, methoxyethyl, naphthyl, phenyl, bromophenyl, chlorophenyl, methoxyphenyl, ethoxyphenyl, tolyl, dimethylphenyl, chorofluorophenyl, methylchlorophenyl, ethylphenyl, phenethyl, benzyl, chlorobenzyl, methylbenzyl, methoxybenzyl, cyanobenzyl, hydroxybenzyl, dichlorobenzyl, dimethoxybenzyl, naphthylmethyl, or (ethoxycarbonyl)ethyl. In a more particular embodiment, R_1 is ethyl, propyl, methoxyethyl, naphthyl, phenethyl, benzyl, chlorobenzyl, methylbenzyl, methoxybenzyl, cyanobenzyl, hydroxybenzyl, dichlorobenzyl, dimethoxybenzyl, naphthylmethyl, or (ethoxycarbonyl)ethyl.

[00103] Most particularly, R_1 is benzyl, chlorobenzyl, methylbenzyl, methoxybenzyl, cyanobenzyl, or hydroxybenzyl. Most particularly, R_1 is benzyl.

R_2

[00104] When considering the compounds of the invention and as will be appreciated by those skilled in the art, the compounds described herein possess a potentially chiral center at the carbon to which R_2 and $R_{2'}$ are attached. The R_2 and $R_{2'}$ groups can be the same or different; if different, the compound is chiral (i.e., has a stereogenic center). When R_2 and $R_{2'}$ are different, in particular embodiments $R_{2'}$ is hydrogen and R_2 is other than hydrogen. The invention contemplates the use of pure enantiomers and mixtures of enantiomers, including racemic mixtures, although the use of a substantially optically pure enantiomer will generally be preferred. The term "substantially optically pure" or "enantiomerically pure" means having at least about 95% of the described enantiomer with no single impurity greater than about 1% and particularly, at least about 97.5% enantiomeric excess. In a particular embodiment, the stereogenic center to which R_2 and $R_{2'}$ are attached is of the R configuration.

[00105] In one embodiment, R_2 is optionally substituted C_1-C_4 alkyl, and $R_{2'}$ is hydrogen or optionally substituted C_1-C_4 alkyl. More particularly, $R_{2'}$ is hydrogen and R_2 is optionally substituted C_1-C_4 alkyl. In a most particular embodiment R_2 is methyl, ethyl, propyl (particularly, c-propyl or i-propyl), butyl (particularly, t-butyl), methylthioethyl, methylthiomethyl, aminobutyl, (CBZ)aminobutyl, cyclohexylmethyl, benzyloxymethyl, methylsulfinylethyl, methylsulfinylmethyl, or hydroxymethyl, and $R_{2'}$ is hydrogen. Especially preferred is when $R_{2'}$ is hydrogen and R_2 is ethyl or propyl (particularly, c-propyl or i-propyl). More particularly, R_2 is i-propyl. More preferred is the embodiment wherein the stereogenic center to which R_2 and $R_{2'}$ is attached is of the R configuration.

[00106] In another embodiment, both R_2 and $R_{2'}$ are hydrogen.

R_4

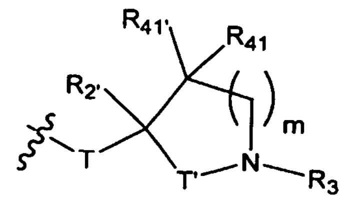
[00107] When considering the compounds of Formula I, in a particular embodiment R_4 is hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted heteroaryl-, optionally substituted aralkyl-, or optionally substituted heteroaralkyl- (especially optionally substituted aryl- or optionally substituted aryl-C_1-C_4-alkyl-).

[00108] In another embodiment, R_4 and R_5 taken together with the carbon and nitrogen to which they are bound, respectively, form an optionally substituted 5- to 7-heterocyclic membered ring.

R_2 taken together with R_7

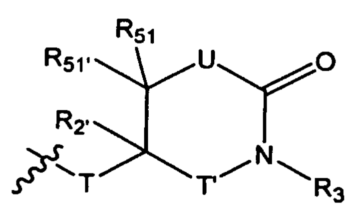
[00109] In another embodiment, R_2 and R_7 taken together form a 5- to 12-membered ring which optionally incorporates from one to two additional heteroatoms, selected from N, O, and S in the heterocycle ring and can optionally be substituted one or more of the following groups: hydroxyl, halogen (particularly chloro or fluoro), optionally substituted C_1-C_4 alkyl- (particularly methyl-), C_1-C_4 alkoxy (particularly methoxy), cyano, amino, substituted amino, oxo, or carbamyl.

[00110] In a particular embodiment, R_2 and R_7 taken together form an optionally substituted ring of the formula:



wherein R_{41} and $R_{41'}$ are independently hydrogen, alkyl, aryl, aralkyl, heteroaryl, substituted alkyl, substituted aryl, substituted aralkyl, or substituted heteroaryl; m is 0, 1, 2, or 3; and T, T', R_3, and $R_{2'}$ are as defined above. In a more particular embodiment, R_{41} is hydrogen. In another particular embodiment, both R_{41} and $R_{41'}$ are hydrogen. See, e.g., PCT application number PCT/US03/30788, filed September 30, 2003, which is incorporated herein by reference for all purposes.

[00111] In another embodiment, R_2 and R_7 taken together form an optionally substituted ring of the formula:



wherein R_3, $R_{2'}$, T, and T' are as defined above; R_{51} and $R_{51'}$ are independently hydrogen, alkyl, aryl, aralkyl, heteroaryl, substituted alkyl, substituted aryl, substituted aralkyl or substituted heteroaryl; U is a covalent bond, CR'R" or NR'"; R' and R" are independently hydrogen, hydroxy, amino, optionally substituted aryl, optionally

substituted alkylamino, optionally substituted alkyl or optionally substituted alkoxy; or R''' is hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, or optionally substituted heteroaralkyl, provided that U and T' are not both covalent bonds.

[00112] In a particular embodiment, R_{51} is hydrogen or optionally substituted lower alkyl; more particularly, R_{51} is hydrogen. In another embodiment, $R_{51'}$ is hydrogen or optionally substituted lower alkyl; more particularly, $R_{51'}$ is hydrogen.

[00113] In one embodiment, U is CR'R" where R' and/or R" are hydrogen. In another embodiment, U is NR''' where R''' is hydrogen or optionally substituted alkyl. More particularly, R''' is hydrogen or optionally substituted amino-lower alkyl. See, e.g., USSN 10/626,012 and PCT/US03/22319, each of which is incorporated herein by reference for all purposes.

R_3

[00114] When considering the compounds of the invention, R_3 is hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaryl-, optionally substituted heteroaralkyl-, -C(O)-R_6, or -$S(O)_2$-R_{6a}. In one embodiment, R_3 is optionally substituted C_1-C_{13} alkyl (especially optionally substituted C_1-C_4 alkyl); optionally substituted aralkyl (especially optionally substituted benzyl or naphthylmethyl-); or optionally substituted heteroaralkyl. More particularly, R_3 is benzyl or benzyl substituted with one or more of the following groups: carboxy, alkoxycarbonyl cyano, halo, C_1-C_4 alkyl-, C_1-C_4 alkoxy, nitro, methylenedioxy, or trifluoromethyl. In another embodiment, and as described below, R_3 is –C(O)R_6. In yet another embodiment, and as described below R_3 is –SO_2R_{6a}

R_5

[00115] When considering the compound of Formula I, in a particular embodiment, R_5 is hydrogen, halogen, optionally substituted alkyl-, optionally substituted alkoxy, hydroxyl-, nitro, cyano, dialkylamino, alkylsulfonyl-, alkylsulfonamido, alkylsulfanyl-, carboxyalkyl-, carboxamido, aminocarbonyl-, optionally substituted aryl-, optionally substituted aralkyl-, optionally substituted heteroaralkyl- or optionally substituted heteroaryl-. In a particular embodiment, R_5 is

hydrogen, halogen, hydroxyl-, lower-alkyl- (particularly methyl-), lower-alkoxy (particularly methoxy) or cyano.

R_6 Groups

[00116] When considering the compounds of the invention wherein R_3 is –C(O)R_6, in a particular embodiment R_6 is optionally substituted C_1-C_8 alkyl, optionally substituted aryl-C_1-C_4-alkyl-, optionally substituted heteroaryl-C_1-C_4-alkyl-, optionally substituted heteroaryl, optionally substituted aryl, R_{11}O- or R_{12}-NH-; R_{11} is optionally substituted C_1-C_8 alkyl or optionally substituted aryl; and R_{12} is hydrogen, optionally substituted C_1-C_8 alkyl or optionally substituted aryl.

Particular R_6 are optionally substituted C_1-C_8 alkyl, optionally substituted aryl-C_1-C_4-alkyl-, optionally substituted heteroaryl-C_1-C_4-alkyl-, optionally substituted heteroaryl, or optionally substituted aryl. In a more particular embodiment, R_6 is phenyl;

phenyl substituted with one or more of the following substituents: halo; C_1-C_4 alkyl; C_1-C_4 alkyl substituted with hydroxy (e.g., hydroxymethyl); C_1-C_4 alkoxy; C_1-C_4 alkyl substituted with C_1-C_4 alkoxy, halo, nitro, formyl, carboxy, cyano, methylenedioxy, ethylenedioxy, acyl (e.g., acetyl), -N-acyl (e.g., N-acetyl) or trifluoromethyl;

benzyl;

phenoxymethyl-;

halophenoxymethyl-;

phenylvinyl-;

heteroaryl-;

heteroaryl- substituted with C_1-C_4 alkyl or C_1-C_4 alkyl substituted with halo (e.g., CF_3);

C_1-C_4 alkyl substituted with C_1-C_4 alkoxy-; or benzyloxymethyl-.

[00117] In a most particular embodiment, R_6 is phenyl, halophenyl, dihalophenyl, cyanophenyl, halo(trifluoromethyl)phenyl, hydroxymethyl-phenyl, methoxymethylphenyl, methoxyphenyl, ethoxyphenyl, carboxyphenyl, formylphenyl, ethylphenyl, tolyl, methylenedioxyphenyl, ethylenedioxyphenyl, methoxychlorophenyl, methylhalophenyl, trifluoromethylphenyl, furanyl, C_1-C_4 alkyl substituted furanyl, trifluoromethylfuranyl, C_1-C_4 alkyl substituted

trifluoromethylfuranyl, benzofuranyl, thiophenyl, C_1-C_4 alkyl substituted thiophenyl, benzothiophenyl, benzothiadiazolyl, pyridinyl, indolyl, methylpyridinyl, trifluoromethylpyridinyl, pyrrolyl, quinolinyl, picolinyl, pyrazolyl, C_1-C_4 alkyl substituted pyrazolyl, N-methyl pyrazolyl, C_1-C_4 alkyl substituted N-methyl pyrazolyl, C_1-C_4 alkyl substituted pyrazinyl, C_1-C_4 alkyl substituted isoxazolyl, benzoisoxazolyl, morpholinomethyl, methylthiomethyl, methoxymethyl, N-methyl imidazolyl, or imidazolyl. Yet more particularly, R_6 is optionally substituted phenyl (especially, tolyl, halophenyl, methylhalophenyl, hydroxymethyl-phenyl, halo(trifluoromethyl)phenyl-, methylenedioxyphenyl, formylphenyl or cyanophenyl).

[00118] In a more particular embodiment, when R_6 is $R_{11}NH$-, R_{11} is hydrogen, C_1-C_4 alkyl; cyclohexyl; phenyl; or phenyl substituted with halo, trifluoromethyl, C_1-C_4 alkyl, C_1-C_4 alkoxy, or C_1-C_4 alkylthio-.

[00119] In a most particular embodiment, when R_6 is $R_{11}NH$-, R_{11} is hydrogen, isopropyl, butyl, cyclohexyl, phenyl, bromophenyl, dichlorophenyl, methoxyphenyl, ethylphenyl, tolyl, trifluoromethylphenyl or methylthio-phenyl.

[00120] In an embodiment, wherein R_6 is R_9O-, R_9 is optionally substituted C_1-C_8 alkyl or optionally substituted aryl.

R_{6a}Groups

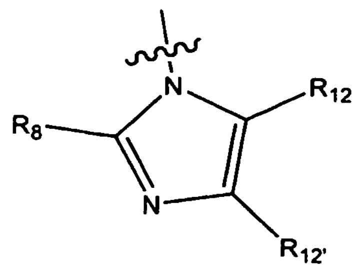
[00121] In one embodiment, when R_3 is $-SO_2R_{6a}$, R_{6a} is C_1-C_{13} alkyl; phenyl; naphthyl; phenyl substituted with halo, lower alkyl, lower alkoxy, cyano, nitro, methylenedioxy, or trifluoromethyl; biphenylyl or heteroaryl. More particularly, R_{6a} is phenyl substituted with halo, lower alkyl, lower alkoxy, cyano, nitro, methylenedioxy, or trifluoromethyl or naphthyl.

R_3 taken together with R_7

[00122] When considering the compounds of the invention, in one embodiment, R_3 taken together with R_7, and the nitrogen to which they are bound, form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates from one to two additional heteroatoms, selected from N, O, and S in the heterocycle ring.

[00123] In a particular embodiment, R_3 taken together with R_7 and the nitrogen to which they are bound, forms an optionally substituted imidazolyl ring of the

formula:



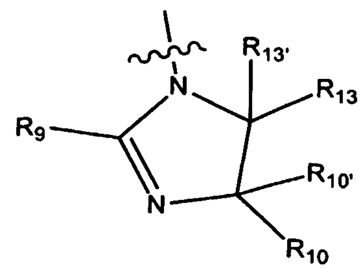
wherein

R_8 is hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaralkyl, optionally substituted aralkoxy, optionally substituted heteroaralkoxy, or optionally substituted heteroaryl; and

R_{12} and $R_{12'}$ are independently hydrogen, optionally substituted alkyl, optionally substituted aryl, or optionally substituted aralkyl.

[00124] More particularly, when R_3 taken together with R_7 and the nitrogen to which they are bound, forms an optionally substituted imidazolyl ring, R_8 is aryl (especially phenyl), substituted aryl (especially lower alkyl-, lower alkoxy-, and/or halo-substituted phenyl), aralkyl (especially benzyl or phenylvinyl), heteroaryl, substituted heteroaryl, heteroaralkyl, aralkoxy (especially phenoxy lower alkyl), heteroaralkoxy, substituted aralkyl (especially substituted benzyl or substituted styrenyl), substituted heteroaralkyl, substituted aralkoxy (especially substituted phenoxy lower alkyl), or substituted heteroaralkoxy. See, e.g., USSN 10/435,069 and PCT/US03/14787, each of which is incorporated herein by reference.

[00125] In another particular embodiment, R_3 taken together with R_7 forms an optionally substituted imidazolinyl ring of the formula:



wherein,

R_9 is hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, optionally substituted heteroaralkyl, optionally substituted aralkoxy, or optionally substituted heteroaralkoxy; and

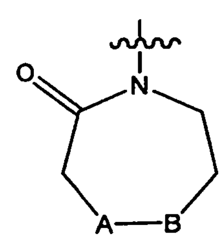
R_{10}, $R_{10'}$, R_{13}, and $R_{13'}$ are independently hydrogen, optionally substituted alkyl, optionally substituted aryl, or optionally substituted aralkyl.

[00126] When R_3 taken together with R_7 forms an optionally substituted imidazolinyl ring, in a particular embodiment, R_9 is aryl (especially phenyl), substituted aryl (especially lower alkyl-, lower alkoxy-, and/or halo-substituted phenyl), aralkyl (especially benzyl or phenylvinyl), heteroaryl, substituted heteroaryl, heteroaralkyl, aralkoxy (especially phenoxy lower alkyl), heteroaralkoxy, substituted aralkyl (especially substituted benzyl or substituted styrenyl), substituted heteroaralkyl, substituted aralkoxy (especially substituted phenoxy lower alkyl), or substituted heteroaralkoxy.

[00127] When R_3 taken together with R_7 forms an optionally substituted imidazolinyl ring, more particularly, R_{10} is hydrogen or optionally substituted lower alkyl, and $R_{10'}$ is hydrogen or optionally substituted lower alkyl.

[00128] In another embodiment, R_3 taken together with R_7 forms an optionally substituted diazepinone ring of the formula:

[00129]

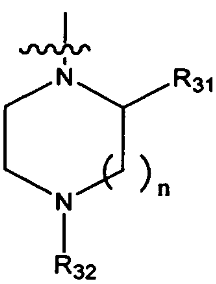


wherein A and B are each independently $C(R_{20})(R_{21})$, $N(R_{22})$, O or S, wherein R_{20} and R_{21} are each independently hydrogen, optionally substituted alkyl optionally substituted aryl or optionally substituted heteroaryl; and R_{22} is H, optionally substituted alkyl, optionally substituted aralkyl, optionally substituted heteroaralkyl, optionally substituted alkylcarbonyl, optionally substituted arylcarbonyl, optionally substituted heteroarylcarbonyl, optionally substituted aralkylcarbonyl, optionally substituted heteroaralkylcarbonyl, optionally substituted alkoxycarbonyl, optionally substituted aryloxycarbonyl, optionally substituted heteroaryloxycarbonyl, optionally substituted aralkyloxycarbonyl, or optionally substituted heteroaralkyloxycarbonyl. In a more particular embodiment, the diazepinone ring is further substituted with one or more of the following groups: optionally substituted alkyl, optionally substituted aryl, optionally substituted aralkyl, optionally substituted heteroaryl, and optionally substituted heteroaralkyl.

[00130] In yet another embodiment of the compounds of Formula I, one of A or B is $C(R_{20})(R_{21})$, wherein R_{20} and R_{21} are each independently hydrogen or C_1-C_4 alkyl, and the other of A or B is $N(R_{22})$, where R_{22} is H, C_1-C_4 alkyl, optionally substituted aralkyl, optionally substituted heteroaralkyl, C_1-C_6 alkylcarbonyl, optionally substituted arylcarbonyl, optionally substituted heteroarylcarbonyl, optionally substituted aralkylcarbonyl, optionally substituted heteroaralkylcarbonyl, C_1-C_6 alkoxycarbonyl, optionally substituted aryloxycarbonyl, optionally substituted heteroaryloxycarbonyl, optionally substituted aralkyloxycarbonyl, or optionally substituted heteroaralkyloxycarbonyl, where the optionally substituted aryl or heteroaryl groups or moieties are unsubstituted or substituted with one or more substituents chosen from C_1-C_4 alkyl, C_1-C_4 haloalkyl, C_1-C_4 alkoxy, C_1-C_4 haloalkoxy, amino, C_1-C_4 alkylamino, di-C_1-C_4 alkylamino, carboxy, C_1-C_4 alkylcarbonyloxy, C_1-C_4 alkoxycarbonyl, carboxamido, C_1-C_4 alkylcarboxamido, aminocarbonyl, C_1-C_4 alkylaminocarbonyl, di-C_1-C_4 alkylaminocarbonyl, cyano, C_1-C_4 alkylcarbonyl, halogen, hydroxyl, mercapto and nitro. In another embodiment, A is $C(R_{20})(R_{21})$, wherein R_{20} and R_{21} are each H or C_1-C_4 alkyl, and B is $N(R_{22})$, where R_{22} is H, C_1-C_4 alkyl, aralkyl, heteroaralkyl, C_1-C_6 alkylcarbonyl, arylcarbonyl, or heteroarylcarbonyl. In specific embodiments of the compounds of Formula I, A is CH_2, and B is $N(R_{22})$, where R_{22} is

H, methyl, benzyl or acetyl (-C(O)methyl). See, e.g., USSN 60/435,001, which is incorporated herein by reference for all purposes.

[00131] In another embodiment, R_3 taken together with R_7 forms an optionally substituted piperazine- or diazepam of the formula:



R_{31} and R_{32} are independently hydrogen, optionally substituted alkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aralkyl, or optionally substituted heteroaralkyl; and n is 1 or 2. More particularly, R_{31} is aryl (especially phenyl), substituted aryl (especially lower alkyl-, lower alkoxy-, and/or halo-substituted phenyl), aralkyl (especially benzyl or phenylvinyl), heteroaralkyl, substituted aralkyl (especially substituted benzyl or substituted phenylvinyl), or substituted heteroaralkyl; R_{32} is hydrogen; and n is 1. See, e.g., USSN 10/644,244 and PCT/US03/26093, each of which is incorporated herein by reference.

R_7

[00132] When considering compounds of the invention, in a particular embodiment, R_7 is hydrogen, optionally substituted C_1-C_{13} alkyl, optionally substituted aryl, optionally substituted aryl-C_1-C_4-alkyl-, optionally substituted heterocyclyl, or optionally substituted heteroaryl-C_1-C_4-alkyl- (especially hydrogen or optionally substituted C_1-C_{13} alkyl).

[00133] More particularly, R_7 is hydrogen, C_1-C_4 alkyl; cyclohexyl; phenyl substituted with hydroxyl, C_1-C_4 alkoxy or C_1-C_4 alkyl; benzyl; or R_{16}-alkylene-, wherein R_{16} is hydroxyl, carboxy, (C_1-C_4 alkoxy)carbonyl-, di(C_1-C_4 alkyl)amino-, (C_1-C_4 alkyl)amino-, amino, (C_1-C_4 alkoxy)carbonylamino-, C_1-C_4 alkoxy-, or optionally substituted N-heterocyclyl- (particularly azetidinyl, morpholinyl, pyridinyl, indolyl, furanyl, pyrrolidinyl, piperidinyl or imidazolyl, each of which can be otionally

substituted).

[00134] In a particular embodiment, R_7 is hydrogen, methyl, ethyl, propyl, butyl, cyclohexyl, carboxyethyl, carboxymethyl, methoxyethyl, hydroxyethyl, hydroxypropyl, dimethylaminoethyl, dimethylaminopropyl, diethylaminoethyl, diethylaminopropyl, aminopropyl, methylaminopropyl, 2,2-dimethyl-3-(dimethylamino)propyl, aminoethyl, aminobutyl, aminopentyl, aminohexyl, isopropylaminopropyl, diisopropylaminoethyl, 1-methyl-4-(diethylamino)butyl, (t-Boc)aminopropyl, hydroxyphenyl, benzyl, methoxyphenyl, methylmethoxyphenyl, dimethylphenyl, tolyl, ethylphenyl, (oxopyrrolidinyl)propyl, (methoxycarbonyl)ethyl, benzylpiperidinyl, pyridinylethyl, pyridinylmethyl, morpholinylethyl morpholinylpropyl, piperidinyl, azetidinylmethyl, azetidinylethyl, azetidinylpropyl pyrrolidinylethyl, pyrrolidinylpropyl, piperidinylmethyl, piperidinylethyl, imidazolylpropyl, imidazolylethyl, (ethylpyrrolidinyl)methyl, (methylpyrrolidinyl)ethyl, (methylpiperidinyl)propyl, (methylpiperazinyl)propyl, furanylmethyl or indolylethyl.

[00135] In another embodiment, R_7 is R_{16}-alkylene-, wherein R_{16} is amino, C_1-C_4 alkylamino-, di(C_1-C_4 alkyl)amino-, C_1-C_4 alkoxy-, hydroxyl, or N-heterocyclyl. Particularly R_{16} is amino. In a particular embodiment, the alkylene moiety of R_{16}-alkylene- has from 1 to 6 carbon atoms.

[00136] More particularly, R_7 is aminoethyl, aminopropyl, aminobutyl, aminopentyl, aminohexyl, methylaminoethyl, methylaminopropyl, methylaminobutyl, methylaminopentyl, methylaminohexyl, dimethylaminoethyl, dimethylaminopropyl, dimethylaminobutyl, dimethylaminopentyl, dimethylaminohexyl, ethylaminoethyl, ethylaminopropyl, ethylaminobutyl, ethylaminopentyl, ethylaminohexyl, diethylaminoethyl, diethylaminopropyl, diethylaminobutyyl, diethylaminopentyl, or diethylaminohexyl, most particularly aminopropyl.

Salt Forms

[00137] The present invention includes pharmaceutically acceptable acid addition salts of the compounds of Formula I. Acid addition salts of the present compounds are prepared in a standard manner in a suitable solvent from the parent compound and an excess of an acid, such as hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, maleic, succinic or methanesulfonic.

[00138] The salts and/or solvates of the compounds of the Formula I which are not pharmaceutically acceptable can be useful as intermediates in the preparation of pharmaceutically acceptable salts and/or solvates of compounds of Formula I or the compounds of the Formula I themselves, and as such form another aspect of the present invention.

Particular Subgenus

[00139] When considering the compounds of the invention, in a particular embodiment,

T and T' are each a covalent bond;

X is $-NR_4-$;

R_1 is benzyl, chlorobenzyl, methylbenzyl, methoxybenzyl, cyanobenzyl, or hydroxybenzyl (especially, benzyl);

$R_{2'}$ is hydrogen;

R_2 is optionally substituted C_1-C_4 alkyl (especially wherein the stereogenic center to which R_2 and $R_{2'}$ is attached is of the R configuration);

R_3 is $-C(O)R_6$;

R_4 is hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted heteroaryl-, optionally substituted aralkyl-, or optionally substituted heteroaralkyl- (especially optionally substituted aryl- or optionally substituted aryl-C_1-C_4-alkyl-);

R_5 is hydrogen, halogen, hydroxyl-, lower-alkyl- (particularly methyl-), lower-alkoxy (particularly methoxy) or cyano;

R_6 is optionally substituted phenyl (especially, tolyl, halophenyl, methylhalophenyl, hydroxymethyl-phenyl, halo(trifluoromethyl)phenyl-, methylenedioxyphenyl, formylphenyl or cyanophenyl);

R_7 is R_{16}-alkylene-; and

R_{16} is amino, C_1-C_4 alkylamino-, di(C_1-C_4 alkyl)amino-, C_1-C_4 alkoxy-, hydroxyl, or N-heterocyclyl.

[00140] When considering the compounds of the invention, in a particular embodiment,

T and T' are each a covalent bond;

X is $-NR_4-$;

R_1 is benzyl, chlorobenzyl, methylbenzyl, methoxybenzyl, cyanobenzyl, or hydroxybenzyl (especially, benzyl);

$R_{2'}$ is hydrogen;

R_2 is optionally substituted C_1-C_4 alkyl (especially wherein the stereogenic center to which R_2 and $R_{2'}$ is attached is of the R configuration);

R_3 is –C(O)R_6;

R_4 and R_5 taken together with the carbon and nitrogen to which they are bound, respectively, form an optionally substituted 5- to 7-heterocyclic membered ring;

R_6 is optionally substituted phenyl (especially, tolyl, halophenyl, methylhalophenyl, hydroxymethyl-phenyl, halo(trifluoromethyl)phenyl-, methylenedioxyphenyl, formylphenyl or cyanophenyl);

R_7 is R_{16}-alkylene-; and

R_{16} is amino, C_1-C_4 alkylamino-, di(C_1-C_4 alkyl)amino-, C_1-C_4 alkoxy-, hydroxyl, or N-heterocyclyl.

[00141] When considering the compounds of the invention, in a particular embodiment,

T and T' are each a covalent bond;

X is O;

R_1 is benzyl, chlorobenzyl, methylbenzyl, methoxybenzyl, cyanobenzyl, or hydroxybenzyl (especially, benzyl);

$R_{2'}$ is hydrogen;

R_2 is optionally substituted C_1-C_4 alkyl (especially wherein the stereogenic center to which R_2 and $R_{2'}$ is attached is of the R configuration);

R_3 is –C(O)R_6;

R_5 is hydrogen, halogen, hydroxyl-, lower-alkyl- (particularly methyl-), lower-alkoxy (particularly methoxy) or cyano;

R_6 is optionally substituted phenyl (especially, tolyl, halophenyl, methylhalophenyl, hydroxymethyl-phenyl, halo(trifluoromethyl)phenyl-, methylenedioxyphenyl, formylphenyl or cyanophenyl);

R_7 is R_{16}-alkylene-; and

R_{16} is amino, C_1-C_4 alkylamino-, di(C_1-C_4 alkyl)amino-, C_1-C_4 alkoxy-; hydroxyl, or N-heterocyclyl.

[00142] When considering the compounds of the invention, in a particular embodiment,

T and T' are each a covalent bond;

X is $-NR_4-$;

R_1 is benzyl, chlorobenzyl, methylbenzyl, methoxybenzyl, cyanobenzyl, or hydroxybenzyl (especially, benzyl);

$R_{2'}$ is hydrogen;

R_2 is optionally substituted C_1-C_4 alkyl (especially wherein the stereogenic center to which R_2 and $R_{2'}$ is attached is of the R configuration);

R_3 taken together with R_7, and the nitrogen to which they are bound, form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates one or two additional heteroatoms, chosen from N, O, and S in the heterocycle ring;

R_4 is hydrogen, optionally substituted alkyl-, optionally substituted aryl-, optionally substituted heteroaryl-, optionally substituted aralkyl-, or optionally substituted heteroaralkyl- (especially optionally substituted aryl- or optionally substituted aryl-C_1-C_4-alkyl-); and

R_5 is hydrogen, halogen, hydroxyl-, lower-alkyl- (particularly methyl-), lower-alkoxy (particularly methoxy) or cyano.

[00143] When considering the compounds of the invention, in a particular embodiment,

T and T' are each a covalent bond;

X is $-NR_4-$;

R_1 is benzyl, chlorobenzyl, methylbenzyl, methoxybenzyl, cyanobenzyl, or hydroxybenzyl (especially, benzyl);

$R_{2'}$ is hydrogen;

R_2 is optionally substituted C_1-C_4 alkyl (especially wherein the stereogenic center to which R_2 and $R_{2'}$ is attached is of the R configuration);

R_3 taken together with R_7, and the nitrogen to which they are bound, form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates one or two additional heteroatoms, chosen from N, O, and S in the heterocycle ring; and

R_4 and R_5 taken together with the carbon and nitrogen to which they are

bound, respectively, form an optionally substituted 5- to 7-heterocyclic membered ring.

[00144] When considering the compounds of the invention, in a particular embodiment,

T and T' are each a covalent bond;

X is O;

R_1 is benzyl, chlorobenzyl, methylbenzyl, methoxybenzyl, cyanobenzyl, or hydroxybenzyl (especially, benzyl);

$R_{2'}$ is hydrogen;

R_2 is optionally substituted C_1-C_4 alkyl (especially wherein the stereogenic center to which R_2 and $R_{2'}$ is attached is of the R configuration);

R_3 taken together with R_7, and the nitrogen to which they are bound, form an optionally substituted 5- to 12-membered nitrogen-containing heterocycle, which optionally incorporates one or two additional heteroatoms, chosen from N, O, and S in the heterocycle ring; and

R_5 is hydrogen, halogen, hydroxyl-, lower-alkyl- (particularly methyl-), lower-alkoxy (particularly methoxy) or cyano.

[00145] Particular compounds of the invention are:

N-(3-Amino-propyl)-N-[1-(3-benzyl-2-oxo-2,3-dihydro-oxazol-4-yl)-2-methyl-propyl]-4-methyl-benzamide;

N-(3-Amino-propyl)-N-[1-(3-benzyl-5-bromo-2-oxo-2,3-dihydro-oxazol-4-yl)-2-methyl-propyl]-4-methyl-benzamide;

N-(3-Amino-propyl)-N-[1-(3-benzyl-2-oxo-1-phenyl-2,3-dihydro-1H-imidazol-4-yl)-2-methyl-propyl]-4-methyl-benzamide;

N-(3-Amino-propyl)-N-[1-(3-benzyl-2-oxo-5-phenyl-2,3-dihydro-oxazol-4-yl)-2-methyl-propyl]-4-methyl-benzamide; and

N-(3-Amino-propyl)-N-[1-(3-benzyl-5-methyl-2-oxo-2,3-dihydro-oxazol-4-yl)-2-methyl-propyl]-4-methyl-benzamide.

Utility, Testing and Administration

General Utility

[00146] Once made, the compounds of the invention find use in at least one of a variety of applications involving alteration of mitosis. As will be appreciated by

those skilled in the art, mitosis can be altered in a variety of ways; that is, one can affect mitosis either by increasing or decreasing the activity of a component in the mitotic pathway. Stated differently, mitosis can be affected (e.g., disrupted) by disturbing equilibrium, either by inhibiting or activating certain components. Similar approaches can be used to alter meiosis.

[00147] In a particular embodiment, the compounds of the invention are used to inhibit mitotic spindle formation, thus causing prolonged cell cycle arrest in mitosis. By "inhibit" in this context is meant decreasing or interfering with mitotic spindle formation or causing mitotic spindle dysfunction. By "mitotic spindle formation" herein is meant organization of microtubules into bipolar structures by mitotic kinesins. By "mitotic spindle dysfunction" herein is meant mitotic arrest and monopolar spindle formation.

[00148] The compounds of the invention are useful to bind to, and/or inhibit the activity of, a mitotic kinesin, KSP. In one embodiment, the KSP is human KSP, although the compounds can be used to bind to or inhibit the activity of KSP kinesins from other organisms. In this context, "inhibit" means either increasing or decreasing spindle pole separation, causing malformation, i.e., splaying, of mitotic spindle poles, or otherwise causing morphological perturbation of the mitotic spindle. Also included within the definition of KSP for these purposes are variants and/or fragments of KSP. See U.S. Patent 6,437,115, hereby incorporated by reference in its entirety. The compounds of the invention have been shown to have specificity for KSP. However, the present invention includes the use of the compounds to bind to or modulate other mitotic kinesins.

[00149] The compounds of the invention are used to treat cellular proliferation diseases. Such disease states which can be treated by the compounds, compositions and methods provided herein include, but are not limited to, cancer (further discussed below), autoimmune disease, fungal disorders, arthritis, graft rejection, inflammatory bowel disease, cellular proliferation induced after medical procedures, including, but not limited to, surgery, angioplasty, and the like. Treatment includes inhibiting cellular proliferation. It is appreciated that in some cases the cells may not be in an abnormal state and still require treatment. Thus, in one embodiment, the invention herein includes application to cells or individuals afflicted or subject to impending affliction with any one of these disorders or states.

[00150] The compounds, pharmaceutical formulations and methods provided herein are particularly deemed useful for the treatment of cancer including solid tumors such as skin, breast, brain, cervical carcinomas, testicular carcinomas, etc. More particularly, cancers that can be treated include, but are not limited to:

- Cardiac: sarcoma (angiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma), myxoma, rhabdomyoma, fibroma, lipoma and teratoma;
- Lung: bronchogenic carcinoma (squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma), alveolar (bronchiolar) carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hamartoma, mesothelioma;
- Gastrointestinal: esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel (adenocarcinoma, lymphoma, carcinoid tumors, Karposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma);
- Genitourinary tract: kidney (adenocarcinoma, Wilm's tumor [nephroblastoma], lymphoma, leukemia), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma), testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma);
- Liver: hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, hemangioma;
- Bone: osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), multiple myeloma, malignant giant cell tumor chordoma, osteochronfroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma and giant cell tumors;
- Nervous system: skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), meninges (meningioma, meningiosarcoma, gliomatosis), brain

(astrocytoma, medulloblastoma, glioma, ependymoma, germinoma [pinealoma], glioblastoma multiform, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors), spinal cord neurofibroma, meningioma, glioma, sarcoma);

- Gynecological: uterus (endometrial carcinoma), cervix (cervical carcinoma, pre-tumor cervical dysplasia), ovaries (ovarian carcinoma [serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma], granulosa-thecal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma (embryonal rhabdomyosarcoma], fallopian tubes (carcinoma);
- Hematologic: blood (myeloid leukemia [acute and chronic], acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, myelodysplastic syndrome), Hodgkin's disease, non-Hodgkin's lymphoma [malignant lymphoma];
- Skin: malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Karposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, keloids, psoriasis; and
- Adrenal glands: neuroblastoma.

As used herein, treatment of cancer includes treatment of cancerous cells, including cells afflicted by any one of the above-identified conditions. Thus, the term "cancerous cell" as provided herein, includes a cell afflicted by any one of the above identified conditions.

[00151] Another useful aspect of the invention is a kit having a compound, salt or solvate of Formula I and a package insert or other labeling including directions treating a cellular proliferative disease by administering an effective amount of the compound, salt or solvate. The compound, salt or solvate of Formula I in the kits of the invention is particularly provided as one or more doses for a course of treatment for a cellular proliferative disease, each dose being a pharmaceutical formulation including a pharmaceutical excipient and a compound, salt or solvate of Formula I.

Testing

[00152] For assay of KSP-modulating activity, generally either KSP or a compound according to the invention is non-diffusably bound to an insoluble support

having isolated sample receiving areas (e.g., a microtiter plate, an array, etc.). The insoluble support can be made of any composition to which the sample can be bound, is readily separated from soluble material, and is otherwise compatible with the overall method of screening. The surface of such supports can be solid or porous and of any convenient shape. Examples of suitable insoluble supports include microtiter plates, arrays, membranes and beads. These are typically made of glass, plastic (e.g., polystyrene), polysaccharides, nylon or nitrocellulose, Teflon™, etc. Microtiter plates and arrays are especially convenient because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples. The particular manner of binding of the sample is not crucial so long as it is compatible with the reagents and overall methods of the invention, maintains the activity of the sample and is nondiffusable. Particular methods of binding include the use of antibodies (which do not sterically block either the ligand binding site or activation sequence when the protein is bound to the support), direct binding to "sticky" or ionic supports, chemical crosslinking, the synthesis of the protein or agent on the surface, etc. Following binding of the sample, excess unbound material is removed by washing. The sample receiving areas can then be blocked through incubation with bovine serum albumin (BSA), casein or other innocuous protein or other moiety.

[00153] The compounds of the invention can be used on their own to inhibit the activity of a mitotic kinesin, particularly KSP. In one embodiment, a compound of the invention is combined with KSP and the activity of KSP is assayed. Kinesin (including KSP) activity is known in the art and includes one or more kinesin activities. Kinesin activities include the ability to affect ATP hydrolysis; microtubule binding; gliding and polymerization/depolymerization (effects on microtubule dynamics); binding to other proteins of the spindle; binding to proteins involved in cell-cycle control; serving as a substrate to other enzymes, such as kinases or proteases; and specific kinesin cellular activities such as spindle pole separation.

[00154] Methods of performing motility assays are well known to those of skill in the art. (See e.g., Hall, et al. (1996), Biophys. J., 71: 3467-3476, Turner et al., 1996, AnaL Biochem. 242 (1):20-5; Gittes et al., 1996, Biophys. J. 70(l): 418-29; Shirakawa et al., 1995, J. Exp. BioL 198: 1809-15; Winkelmann et al., 1995, Biophys. J. 68: 2444-53; Winkelmann et al., 1995, Biophys. J. 68: 72S.)

[00155] Methods known in the art for determining ATPase hydrolysis activity

also can be used. Suitably, solution based assays are utilized. U.S. Patent 6,410,254, hereby incorporated by reference in its entirety, describes such assays. Alternatively, conventional methods are used. For example, P_i release from kinesin can be quantified. In one embodiment, the ATPase hydrolysis activity assay utilizes 0.3 M PCA (perchloric acid) and malachite green reagent (8.27 mM sodium molybdate II, 0.33 mM malachite green oxalate, and 0.8 mM Triton X-1 00). To perform the assay, 10 μL of the reaction mixture is quenched in 90 μL of cold 0.3 M PCA. Phosphate standards are used so data can be converted to mM inorganic phosphate released. When all reactions and standards have been quenched in PCA, 100 μL of malachite green reagent is added to the relevant wells in e.g., a microtiter plate. The mixture is developed for 10-15 minutes and the plate is read at an absorbance of 650 nm. If phosphate standards were used, absorbance readings can be converted to mM P_i and plotted over time. Additionally, ATPase assays known in the art include the luciferase assay.

[00156] ATPase activity of kinesin motor domains also can be used to monitor the effects of agents and are well known to those skilled in the art. In one embodiment ATPase assays of kinesin are performed in the absence of microtubules. In another embodiment, the ATPase assays are performed in the presence of microtubules. Different types of agents can be detected in the above assays. In a one embodiment, the effect of an agent is independent of the concentration of microtubules and ATP. In another embodiment, the effect of the agents on kinesin ATPase can be decreased by increasing the concentrations of ATP, microtubules or both. In yet another embodiment, the effect of the agent is increased by increasing concentrations of ATP, microtubules or both.

[00157] Compounds that inhibit the biochemical activity of KSP in vitro can then be screened in vivo. In vivo screening methods include assays of cell cycle distribution, cell viability, or the presence, morphology, activity, distribution, or number of mitotic spindles. Methods for monitoring cell cycle distribution of a cell population, for example, by flow cytometry, are well known to those skilled in the art, as are methods for determining cell viability. See for example, U.S. Patent 6,437,115, hereby incorporated by reference in its entirety. Microscopic methods for monitoring spindle formation and malformation are well known to those of skill in the art (see, e.g., Whitehead and Rattner (1998), J. Cell Sci. 111:2551-61; Galgio et al, (1996) J.

Cell Biol., 135:399-414), each incorporated herein by reference in its entirety.

[00158] The compounds of the invention inhibit the KSP kinesin. One measure of inhibition is IC_{50}, defined as the concentration of the compound at which the activity of KSP is decreased by fifty percent relative to a control. Preferred compounds have IC_{50}'s of less than about 1 mM, with preferred embodiments having IC_{50}'s of less than about 100 μM, with more preferred embodiments having IC_{50}'s of less than about 10 μM, with particularly preferred embodiments having IC_{50}'s of less than about 1 μM, and especially preferred embodiments having IC_{50}'s of less than about 100 nM, and with the most preferred embodiments having IC_{50}'s of less than about 10 nM. Measurement of IC_{50} is done using an ATPase assay such as described herein.

[00159] Another measure of inhibition is K_i. For compounds with IC_{50}'s less than 1 μM, the K_i or K_d is defined as the dissociation rate constant for the interaction of the compounds described herein with KSP. Preferred compounds have K_i's of less than about 100 μM, with preferred embodiments having K_i's of less than about 10 μM, and particularly preferred embodiments having K_i's of less than about 1 μM and especially preferred embodiments having K_i's of less than about 100 nM, and with the most preferred embodiments having K_i's of less than about 10 nM.

[00160] The K_i for a compound is determined from the IC_{50} based on three assumptions and the Michaelis-Menten equation. First, only one compound molecule binds to the enzyme and there is no cooperativity. Second, the concentrations of active enzyme and the compound tested are known (i.e., there are no significant amounts of impurities or inactive forms in the preparations). Third, the enzymatic rate of the enzyme-inhibitor complex is zero. The rate (i.e., compound concentration) data are fitted to the equation:

$$V = V_{max}E_0 \left[I - \frac{(E_0 + I_0 + Kd) - \sqrt{(E_0 + I_0 + Kd)^2 - 4E_0 I_0}}{2E_0} \right]$$

where V is the observed rate, V_{max} is the rate of the free enzyme, I_0 is the inhibitor concentration, E_0 is the enzyme concentration, and K_d is the dissociation constant of the enzyme-inhibitor complex.

[00161] Another measure of inhibition is GI_{50}, defined as the concentration of

the compound that results in a decrease in the rate of cell growth by fifty percent. Preferred compounds have GI_{50}'s of less than about 1 mM; those having a GI_{50} of less than about 20 μM are more preferred; those having a GI_{50} of less than about 10 μM more so; those having a GI_{50} of less than about 1 μM more so; those having a GI_{50} of less than about 100 nM more so; and those having a GI_{50} of less than about 10 nM even more so. Measurement of GI_{50} is done using a cell proliferation assay such as described herein. Compounds of this class were found to inhibit cell proliferation.

[00162] In vitro potency of small molecule inhibitors is determined, for example, by assaying human ovarian cancer cells (SKOV3) for viability following a 72-hour exposure to a 9-point dilution series of compound. Cell viability is determined by measuring the absorbance of formazon, a product formed by the bioreduction of MTS/PMS, a commercially available reagent. Each point on the dose-response curve is calculated as a percent of untreated control cells at 72 hours minus background absorption (complete cell kill).

[00163] Anti-proliferative compounds that have been successfully applied in the clinic to treatment of cancer (cancer chemotherapeutics) have GI_{50}'s that vary greatly. For example, in A549 cells, paclitaxel GI_{50} is 4 nM, doxorubicin is 63 nM, 5-fluorouracil is 1 μM, and hydroxyurea is 500 μM (data provided by National Cancer Institute, Developmental Therapeutic Program, http://dtp.nci.nih.gov/). Therefore, compounds that inhibit cellular proliferation, irrespective of the concentration demonstrating inhibition, have potential clinical usefulness.

[00164] To employ the compounds of the invention in a method of screening for compounds that bind to KSP kinesin, the KSP is bound to a support, and a compound of the invention is added to the assay. Alternatively, the compound of the invention is bound to the support and KSP is added. Classes of compounds among which novel binding agents can be sought include specific antibodies, non-natural binding agents identified in screens of chemical libraries, peptide analogs, etc. Of particular interest are screening assays for candidate agents that have a low toxicity for human cells. A wide variety of assays can be used for this purpose, including labeled in vitro protein-protein binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, functional assays (phosphorylation assays, etc.) and the like.

[00165] The determination of the binding of the compound of the invention to

KSP can be done in a number of ways. In one embodiment, the compound is labeled, for example, with a fluorescent or radioactive moiety, and binding is determined directly. For example, this can be done by attaching all or a portion of KSP to a solid support, adding a labeled test compound (for example a compound of the invention in which at least one atom has been replaced by a detectable isotope), washing off excess reagent, and determining whether the amount of the label is that present on the solid support.

[00166] By "labeled" herein is meant that the compound is either directly or indirectly labeled with a label which provides a detectable signal, e.g., radioisotope, fluorescent tag, enzyme, antibodies, particles such as magnetic particles, chemiluminescent tag, or specific binding molecules, etc. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin etc. For the specific binding members, the complementary member would normally be labeled with a molecule which provides for detection, in accordance with known procedures, as outlined above. The label can directly or indirectly provide a detectable signal.

[00167] In some embodiments, only one of the components is labeled. For example, the kinesin proteins can be labeled at tyrosine positions using ^{125}I, or with fluorophores. Alternatively, more than one component can be labeled with different labels; using ^{125}I for the proteins, for example, and a fluorophor for the antimitotic agents.

[00168] The compounds of the invention can also be used as competitors to screen for additional drug candidates. "Candidate agent" or "drug candidate" or grammatical equivalents as used herein describe any molecule, e.g., protein, oligopeptide, small organic molecule, polysaccharide, polynucleotide, etc., to be tested for bioactivity. They can be capable of directly or indirectly altering the cellular proliferation phenotype or the expression of a cellular proliferation sequence, including both nucleic acid sequences and protein sequences. In other cases, alteration of cellular proliferation protein binding and/or activity is screened. Screens of this sort can be performed either in the presence or absence of microtubules. In the case where protein binding or activity is screened, particular embodiments exclude molecules already known to bind to that particular protein, for example, polymer structures such as microtubules, and energy sources such as ATP. Particular embodiments of assays herein include candidate agents which do not bind the cellular

proliferation protein in its endogenous native state termed herein as "exogenous" agents. In another embodiment, exogenous agents further exclude antibodies to KSP.

[00169] Candidate agents can encompass numerous chemical classes, though typically they are small organic compounds having a molecular weight of more than 100 and less than about 2,500 daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding and lipophilic binding, and typically include at least an amine, carbonyl-, hydroxyl-, ether, or carboxyl group, generally at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

[00170] Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means. Known pharmacological agents can be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, and/or amidification to produce structural analogs.

[00171] Competitive screening assays can be done by combining KSP and a drug candidate in a first sample. A second sample comprises a compound of the present invention, KSP and a drug candidate. This can be performed in either the presence or absence of microtubules. The binding of the drug candidate is determined for both samples, and a change, or difference in binding between the two samples indicates the presence of a drug candidate capable of binding to KSP and potentially inhibiting its activity. That is, if the binding of the drug candidate is different in the second sample relative to the first sample, the drug candidate is capable of binding to KSP.

[00172] In a particular embodiment, the binding of the candidate agent to KSP

is determined through the use of competitive binding assays. In this embodiment, the competitor is a binding moiety known to bind to KSP, such as an antibody, peptide, binding partner, ligand, etc. Under certain circumstances, there can be competitive binding as between the candidate agent and the binding moiety, with the binding moiety displacing the candidate agent.

[00173] In one embodiment, the candidate agent is labeled. Either the candidate agent, or the competitor, or both, is added first to KSP for a time sufficient to allow binding, if present. Incubations can be performed at any temperature which facilitates optimal activity, typically between 4 and 40°C.

[00174] Incubation periods are selected for optimum activity, but can also be optimized to facilitate rapid high throughput screening. Typically between 0.1 and 1 hour will be sufficient. Excess reagent is generally removed or washed away. The second component is then added, and the presence or absence of the labeled component is followed, to indicate binding.

[00175] In another embodiment, the competitor is added first, followed by the candidate agent. Displacement of the competitor is an indication the candidate agent is binding to KSP and thus is capable of binding to, and potentially inhibiting, the activity of KSP. In this embodiment, either component can be labeled. Thus, for example, if the competitor is labeled, the presence of label in the wash solution indicates displacement by the agent. Alternatively, if the candidate agent is labeled, the presence of the label on the support indicates displacement.

[00176] In an alternative embodiment, the candidate agent is added first, with incubation and washing, followed by the competitor. The absence of binding by the competitor can indicate the candidate agent is bound to KSP with a higher affinity. Thus, if the candidate agent is labeled, the presence of the label on the support, coupled with a lack of competitor binding, can indicate the candidate agent is capable of binding to KSP.

[00177] Inhibition is tested by screening for candidate agents capable of inhibiting the activity of KSP comprising the steps of combining a candidate agent with KSP, as above, and determining an alteration in the biological activity of KSP. Thus, in this embodiment, the candidate agent should both bind to KSP (although this may not be necessary), and alter its biological or biochemical activity as defined herein. The methods include both in vitro screening methods and in vivo screening of

cells for alterations in cell cycle distribution, cell viability, or for the presence, morpohology, activity, distribution, or amount of mitotic spindles, as are generally outlined above.

[00178] Alternatively, differential screening can be used to identify drug candidates that bind to the native KSP, but cannot bind to modified KSP.

[00179] Positive controls and negative controls can be used in the assays. Suitably all control and test samples are performed in at least triplicate to obtain statistically significant results. Incubation of all samples is for a time sufficient for the binding of the agent to the protein. Following incubation, all samples are washed free of non-specifically bound material and the amount of bound, generally labeled agent determined. For example, where a radiolabel is employed, the samples can be counted in a scintillation counter to determine the amount of bound compound.

[00180] A variety of other reagents can be included in the screening assays. These include reagents like salts, neutral proteins, e.g., albumin, detergents, etc which can be used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Also reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., can be used. The mixture of components can be added in any order that provides for the requisite binding.

Administration

[00181] Accordingly, the compounds of the invention are administered to cells. By "administered" herein is meant administration of a therapeutically effective dose of a compound of the invention to a cell either in cell culture or in a patient. By "therapeutically effective dose" herein is meant a dose that produces the effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. As is known in the art, adjustments for systemic versus localized delivery, age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art. By "cells" herein is meant any cell in which mitosis or meiosis can be altered.

[00182] A "patient" for the purposes of the present invention includes both

humans and other animals, particularly mammals, and other organisms. Thus the methods are applicable to both human therapy and veterinary applications. In a particular embodiment the patient is a mammal, and more particularly, the patient is human.

[00183] Compounds of the invention having the desired pharmacological activity can be administered, especially as a pharmaceutically acceptable composition comprising an pharmaceutical excipient, to a patient, as described herein. Depending upon the manner of introduction, the compounds can be formulated in a variety of ways as discussed below. The concentration of therapeutically active compound in the formulation can vary from about 0.1-100 wt.%.

[00184] The agents can be administered alone or in combination with other treatments, i.e., radiation, or other chemotherapeutic agents such as the taxane class of agents that appear to act on microtubule formation or the camptothecin class of topoisomerase I inhibitors. When used, other chemotherapeutic agents can be administered before, concurrently, or after administration of a compound of the present invention. In one aspect of the invention, a compound of the present invention is co-administered with one or more other chemotherapeutic agents. By "co-administer" it is meant that the present compounds are administered to a patient such that the present compounds as well as the co-administered compound can be found in the patient's bloodstream at the same time, regardless when the compounds are actually administered, including simultaneously.

[00185] The administration of the compounds and compositions of the present invention can be done in a variety of ways, including, but not limited to, orally, subcutaneously, intravenously, intranasally, transdermally, intraperitoneally, intramuscularly, intrapulmonary, vaginally, rectally, or intraocularly. In some instances, for example, in the treatment of wounds and inflammation, the compound or composition can be directly applied as a solution or spray.

[00186] Pharmaceutical dosage forms include a compound of Formula I or a pharmaceutically acceptable salt, solvate, or solvate of a salt thereof, and one or more pharmaceutical excipients. As is known in the art, pharmaceutical excipients are secondary ingredients which function to enable or enhance the delivery of a drug or medicine in a variety of dosage forms (e.g.: oral forms such as tablets, capsules, and liquids; topical forms such as dermal, opthalmic, and otic forms; suppositories;

injectables; respiratory forms and the like). Pharmaceutical excipients include inert or inactive ingredients, synergists or chemicals that substantively contribute to the medicinal effects of the active ingredient. For example, pharmaceutical excipients can function to improve flow characteristics, product uniformity, stability, taste, or appearance, to ease handling and administration of dose, for convenience of use, or to control bioavailability. While pharmaceutical excipients are commonly described as being inert or inactive, it is appreciated in the art that there is a relationship between the properties of the pharmaceutical excipients and the dosage forms containing them.

[00187] Pharmaceutical excipients suitable for use as carriers or diluents are well known in the art, and can be used in a variety of formulations. See, e.g., Remington's Pharmaceutical Sciences, 18th Edition, A. R. Gennaro, Editor, Mack Publishing Company (1990); Remington: The Science and Practice of Pharmacy, 20th Edition, A. R. Gennaro, Editor, Lippincott Williams & Wilkins (2000); Handbook of Pharmaceutical Excipients, 3rd Edition, A. H. Kibbe, Editor, American Pharmaceutical Association, and Pharmaceutical Press (2000); and Handbook of Pharmaceutical Additives, compiled by Michael and Irene Ash,Gower (1995), each of which is incorporated herein by reference for all purposes.

[00188] Oral solid dosage forms such as tablets will typically comprise one or more pharmaceutical excipients, which can for example help impart satisfactory processing and compression characteristics, or provide additional desirable physical characteristics to the tablet. Such pharmaceutical excipients can be selected from diluents, binders, glidants, lubricants, disintegrants, colors, flavors, sweetening agents, polymers, waxes or other solubility-retarding materials.

[00189] Compositions for intravenous administration will generally comprise intravenous fluids, i.e., sterile solutions of simple chemicals such as sugars, amino acids or electrolytes, which can be easily carried by the circulatory system and assimilated. Such fluids are prepared with water for injection USP.

[00190] Dosage forms for parenteral administration will generally comprise fluids, particularly intravenous fluids, i.e., sterile solutions of simple chemicals such as sugars, amino acids or electrolytes, which can be easily carried by the circulatory system and assimilated. Such fluids are typically prepared with water for injection USP. Fluids used commonly for intravenous (IV) use are disclosed in Remington, The Science and Practice of Pharmacy [full citation previously provided], and include:

- alcohol, e.g., 5% alcohol (e.g., in dextrose and water ("D/W") or D/W in normal saline solution ("NSS"), including in 5% dextrose and water ("D5/W"), or D5/W in NSS);
- synthetic amino acid such as Aminosyn, FreAmine, Travasol, e.g., 3.5 or 7; 8.5; 3.5, 5.5 or 8.5 % respectively;
- ammonium chloride e.g., 2.14%;
- dextran 40, in NSS e.g., 10% or in D5/W e.g., 10%;
- dextran 70, in NSS e.g., 6% or in D5/W e.g., 6%;
- dextrose (glucose, D5/W) e.g., 2.5-50%;
- dextrose and sodium chloride e.g., 5-20% dextrose and 0.22-0.9% NaCl;
- lactated Ringer's (Hartmann's) e.g., NaCl 0.6%, KCl 0.03%, $CaCl_2$ 0.02%;
- lactate 0.3%;
- mannitol e.g., 5%, optionally in combination with dextrose e.g., 10% or NaCl e.g., 15 or 20%;
- multiple electrolyte solutions with varying combinations of electrolytes, dextrose, fructose , invert sugar Ringer's e.g., NaCl 0.86%, KCl 0.03%, $CaCl_2$ 0.033%;
- sodium bicarbonate e.g., 5%;
- sodium chloride e.g., 0.45, 0.9, 3, or 5%;
- sodium lactate e.g., 1/6 M; and
- sterile water for injection

The pH of such IV fluids can vary, and will typically be from 3.5 to 8 as known in the art.

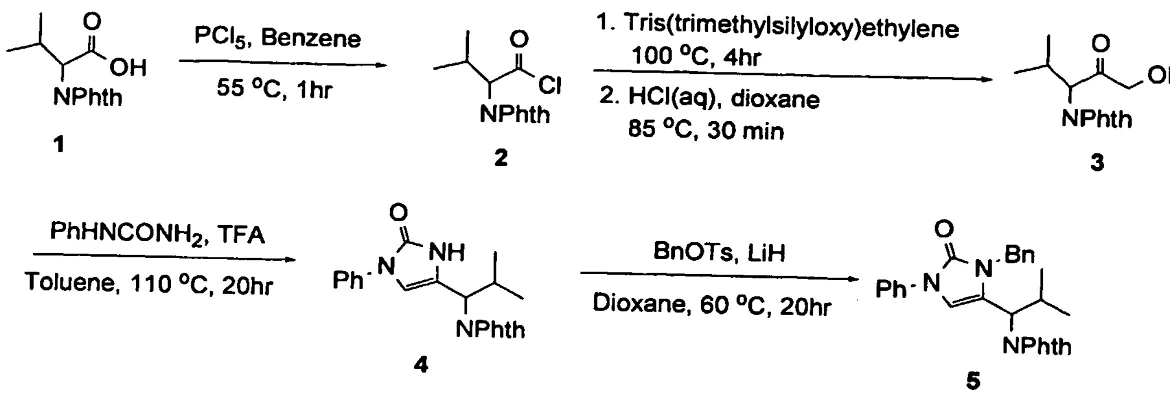
[00191] The compounds, pharmaceutically acceptable salts and solvates of the invention can be administered alone or in combination with other treatments, i.e., radiation, or other therapeutic agents, such as the taxane class of agents that appear to act on microtubule formation or the camptothecin class of topoisomerase I inhibitors. When so-used, other therapeutic agents can be administered before, concurrently (whether in separate dosage forms or in a combined dosage form), or after administration of an active agent of the present invention.

[00192] The following examples serve to more fully describe the manner of using the above-described invention. It is understood that these examples in no way serve to limit the true scope of this invention, but rather are presented for illustrative purposes. All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein.

EXAMPLES

Example 1

Synthesis of Compounds



[00193] A suspension of *N*-phthaloyl-DL-Val-OH (25.0 g, 100 mmol) and phosphorus pentachloride (22.8 g, 110 mmol) in anhydrous benzene (150 mL) was heated to 55 °C for one hour. The resulting solution was cooled to room temperature and the solid was removed by filtration. The organic layer was concentrated *in vacuo* and washed twice with dry toluene. The residue **2** was used in the next step without purification.

[00194] A mixture of *N*-phthaloyl-DL-valinyl chloride **2** (22.5 g, 85 mmol) and 1,1,2-tris(trimethylsilyloxy)ethylene (41.2 g, 170 mmol) was stirred at 100 °C for 4 hours. The resulting solution was cooled to room temperature and treated with a solution of aqueous hydrochloric acid (34 mL, 0.6 M) in dioxane (85 mL). The resulting mixture was then heated to 85 °C for 30 minutes and cooled to room temperature. It was then saturated with sodium chloride (30 g) and extracted with diethyl ether (3 x 100 mL). The combined organic layers were washed with saturated aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated *in vacuo*. The residue **3** (16 g) was determined to be pure enough for the next transformation without purification (^{1}H-NMR and LC/MS (LRMS (MH) *m/z*: 261.27)).

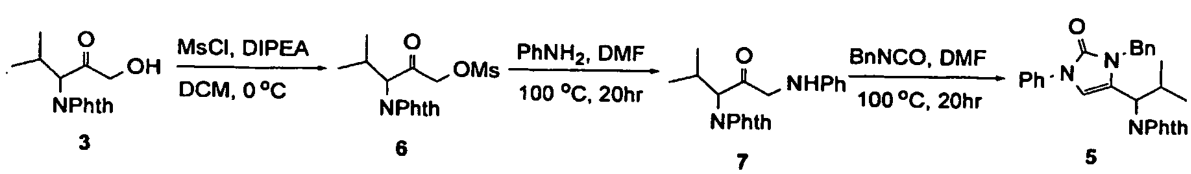
[00195] To a room temperature solution of compound **3** (2.61 g, 10.0 mmol)

and phenylurea (4.1 g, 15.0 mmol) in toluene (30 mL) was added trifluoroacetic acid (10 mL). The resulting solution was sealed and stirred at 110 °C for 20 hours. It was then cooled to room temperature and concentrated *in vacuo*. The residue was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution. The aqueous phase was extracted with ethyl acetate (3 x 80 mL), and the combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, hexane and ethyl acetate), and the desired product **4** (300 mg) was isolated and characterized by ^{1}H-NMR and LC/MS (LRMS (MH) *m/z*: 361.39).

[00196] To a room temperature solution of intermediate **4** (300 mg, 0.8 mmol) in dioxane (10 mL) were added lithium hydride (40 mg) and benzyl *p*-tolunesulfonate (400 mg, 1.5 mmol), successively. The resulting solution was heated to 60 °C for 24 hours. It was then cooled to room temperature and quenched with saturated aqueous sodium bicarbonate solution, and the aqueous phase was extracted with ethyl acetate (3 x 60 mL). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, hexane and ethyl acetate), and the desired product **5** (235 mg) was isolated and characterized by ^{1}H-NMR and LC/MS (LRMS (MH) *m/z*: 451.52).

Example 2

Alternative Preparation of Imidazolones



[00197] To a solution of α-hydroxy ketone **3** (2.61 g, 10.0 mmol) and diisopropylethylamine (2.1 mL, 12.0 mmol) in dichloromethane (100 mL) at 0 °C was added a solution of methanesulfonyl chloride (1.26g, 11.0 mmol) in dichloromethane (20 mL). The resulting solution was stirred at the same temperature for one hour. It was then quenched with saturated aqueous sodium bicarbonate solution, and the aqueous phase was extracted with dichloromethane (3 x 60 mL). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The residue

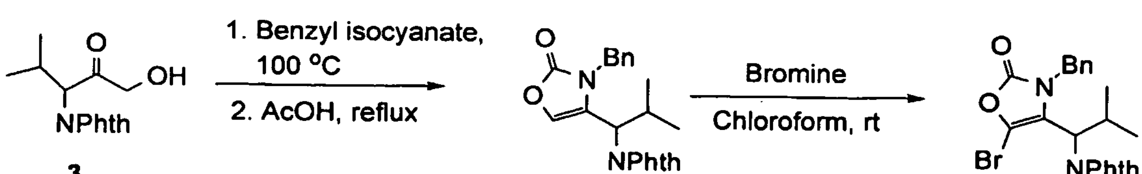
was purified by flash chromatography (silica gel, hexane and ethyl acetate plus 2% triethylamine), and α-mesyloxy ketone **6** (2.7 g) was isolated and characterized by ^{1}H-NMR and LC/MS (LRMS (MH) *m/z* 339.36).

[00198] To a room temperature solution of α-mesyloxy ketone **6** (3.39 g, 10.0 mmol) in N,N-dimethylformamide (50 mL) was added aniline (1.2 mL, 12.0 mmol). The resulting solution was stirred at 100 °C for 20 hours. It was cooled to room temperature and the solvents were removed *in vacuo*. The residue was purified by flash chromatography (silica gel, dichloromethane and methanol), and the desired product **7** (1.5 g) was isolated and characterized by ^{1}H-NMR and LC/MS (LRMS (MH) *m/z* 336.38).

[00199] To a room temperature solution of compound **7** (620 mg, 1.8 mmol) in toluene (20 mL) was added benzyl isocyanate (0.62 mL, 4.6 mmol). The resulting solution was stirred at 110 °C for 20 hours and cooled to room temperature. It was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution. The aqueous phase was extracted with ethyl acetate (3 x 60 mL), and the combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, hexane and ethyl acetate), and the desired product **5** (500 mg) was isolated and characterized by ^{1}H-NMR and LC/MS (LRMS (MH) *m/z*: 451.52).

Example 3

Preparation of Oxazolones



3 8 9

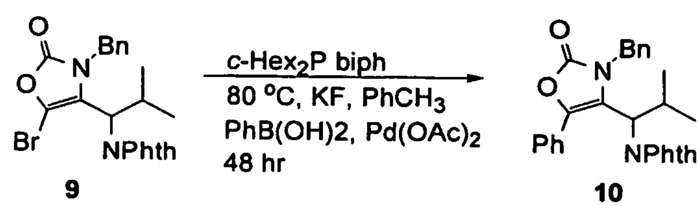
[00200] To a room temperature solution of alcohol **3** (584 mg, 2.34 mmol) in N,N-dimethylformamide (2 mL) was added benzyl isocyanate (414 μL, 3.36 mmol). The resulting solution was stirred at 100 °C for 2 hours under nitrogen and then cooled to room temperature. It was diluted with ethyl acetate (50 mL) and washed

with water (4 x 50 mL) and brine (50 mL). The resulting organic layer was dried over sodium sulfate and concentrated *in vacuo*. The residual oil was purified by flash column chromatography (silica gel, hexanes and ethyl acetate), and the desired intermediate (560 mg) was isolated and characterized by ^{1}H-NMR and LC/MS (LRMS (MH) *m/z*: 394.1).

[00201] A solution of the intermediate above (507 mg, 1.29 mmol) in glacial acetic acid (20 mL) was refluxed for 8 hours and cooled to room temperature. And the resulting solution was concentrated *in vacuo* to provide compound **8** (393 mg), which was characterized by ^{1}H-NMR and LC/MS (LRMS (MH) *m/z*: 377.1) and used in the next step without purification.

[00202] To a room temperature solution of oxazolone **8** (413 mg, 1.10 mmol) in chloroform (5 mL) was added bromine (210 mg, 1.32 mmol). The evolved hydrogen bromide was continually displaced by a free-flowing stream of nitrogen, and the resulting solution was stirred for 1 hour at the same temperature. It was then diluted with dichloromethane (40 mL) and washed with water (10 mL) and aqueous sodium hydrogensulfite solution (10 mL). The organic layers were dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes and ethyl acetate), and the desired product **9** (452 mg) was isolated and characterized by ^{1}H-NMR and LC/MS (LRMS (MH) *m/z*: 457.0).

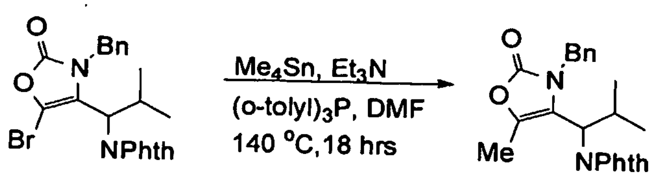
Example 4



[00203] To a 100 mL round bottom flask were added oxazolone **9** (1.41 g, 3.09 mmol), phenylboronic acid (565 mg, 4.64 mmol), palladium(II) acetate (14 mg, 1 mol %), 2-(dicyclohexyl)phosphinobiphenyl (40 mg, 2 mol %) and potassium fluoride (539 mg, 9.27 mmol). The flask was flusheded by nitrogen three times. Toluene (15

mL) was added, and the resulting mixture was then stirred at 110 °C for 48 hours and cooled to room temperature. It was then diluted with diethyl ether (30 mL) and washed with aqueous potassium hydroxide solution (20 mL, 1.0 M). The aqueous phase was extracted with additional diethyl ether (3 x 15 mL), and the combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes and ethyl acetate), and the desired product **10** (334 mg) was isolated and characterized by ^{1}H-NMR and LC/MS (LRMS (MH) *m/z*: 453.1).

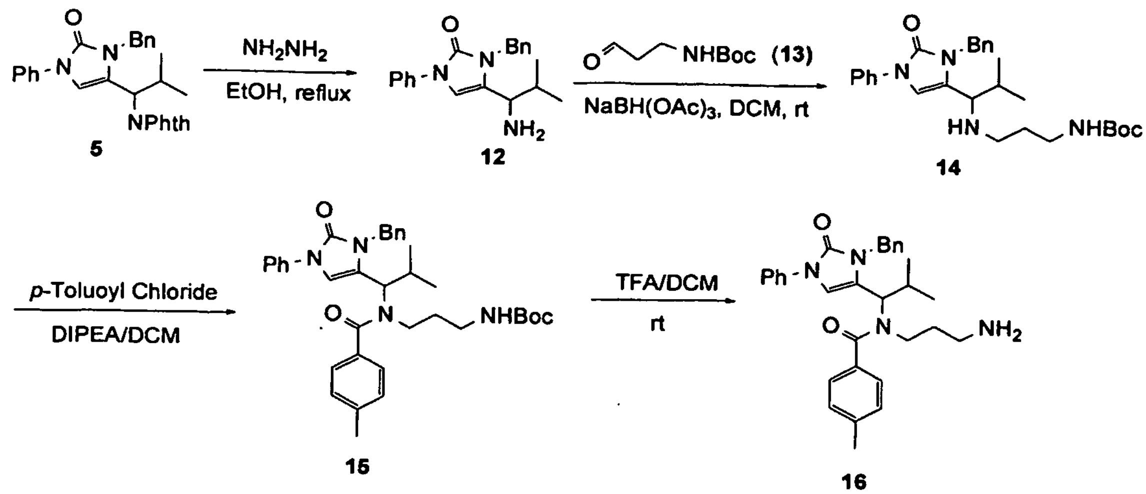
Example 5



[00204] To a thick-walled glass tube containing oxazolone **9** (1.18 g, 2.56 mmol), tri-*o*-tolylphosphine (126 mg, 8 mol %), tetramethyltin (716 μL, 5.17 mmol), and palladium(II) acetate (12 mg, 2 mol %) were added *N, N*-dimethylformamide (5 mL) and triethylamine (1.1 mL, 7.76 mmol). The resulting solution was purged with nitrogen, and the tube was quickly sealed and heated to 115 °C for 18 hours. It was then cooled to room temperature, diluted with ethyl acetate (75 mL), and washed with water (5 x 50 mL) and brine (50 mL). The resulting organic layers were dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes ethyl acetate), and the desired product **11** (653 mg) was isolated and characterized by ^{1}H-NMR and LC/MS (LRMS (MH) *m/z*: 391.1).

Example 6

Preparation of imidazolone derivatives



[00205] To a room temperature solution of compound **5** (520 mg, 1.0 mmol) in ethanol (6 mL) was added a solution of hydrazine in tetrahydrofuran (10 mL, 1 M). The resulting solution was stirred at 55 °C for 20 hours and then cooled to room temperature. The solvents were removed *in vacuo*. The residue was purified by flash chromatography (silica gel, dichloromethane and methanol), and the desired free amine **12** (140 mg) was isolated and characterized by ^{1}H-NMR and LC/MS (LRMS (MH) *m/z*: 321.42).

[00206] To a room temperature solution of free amine **12** (400 mg, 1.23 mmol) in dichloromethane (15 mL) were added sodium triacetoxyborohydride (313 mg, 1.48 mmol) and aldehyde **13** (277 mg, 1.60 mmol), successively. The resulting mixture was stirred at the same temperature under nitrogen for 12 hours. It was then quenched with saturated aqueous sodium bicarbonate solution, and the aqueous phase was extracted with dichloromethane (3 x 60 mL). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, dichloromethane/methanol), and the desired product **14** (452 mg) was isolated and characterized by LC/MS (LRMS (MH) *m/z* 478.63).

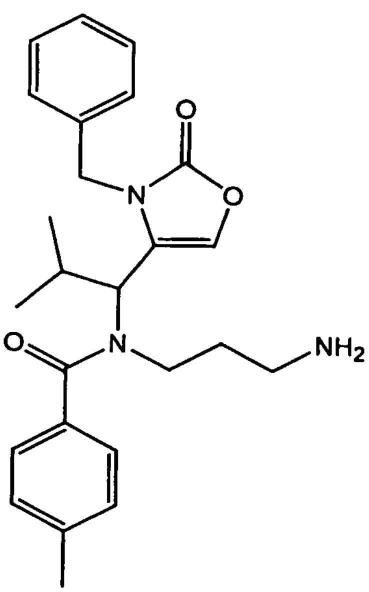
[00207] To a solution of imidazolone **14** (452 mg, 0.95 mmol) in dichloromethane (20 mL) at 0 °C were added diisopropylethylamine (1.0 mL) and *p*-toluoyl chloride (156 mg, 1.0 mmol), successively. The resulting solution was stirred at room temperature under nitrogen overnight. It was quenched with saturated aqueous sodium bicarbonate solution, and the aqueous phase was extracted with ethyl acetate

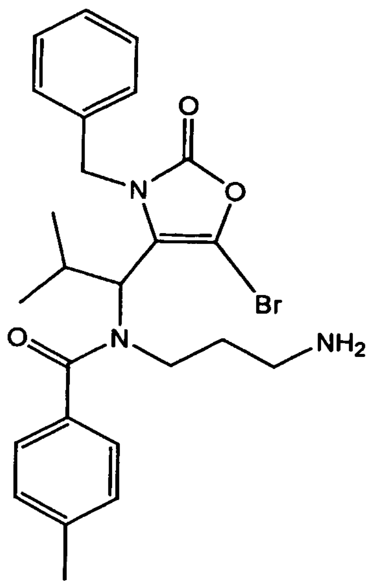
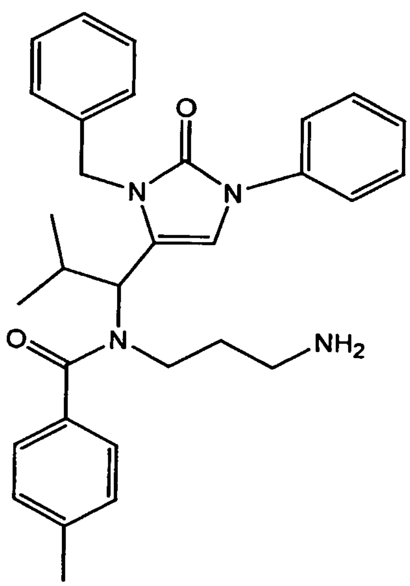
(4 x 50 mL). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, dichloromethane and methanol), and the desired product **15** (306 mg) was isolated and characterized by ^{1}H-NMR and LC/MS (LRMS (MH) *m/z:* 596.76).

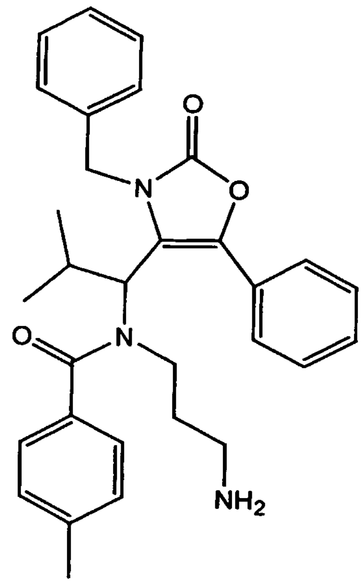
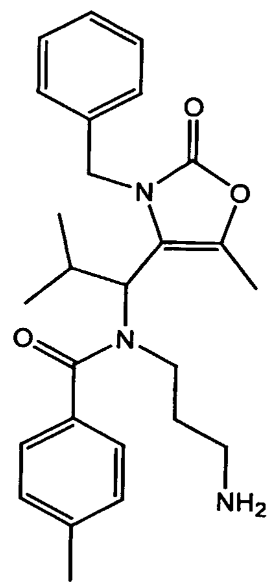
[00208] To a solution of imidazolone **15** (306 mg, 0.51 mmol) in dichloromethane (12 mL) at 0 °C was added trifluoroacetic acid (4 mL). The resulting solution was stirred at room temperature for 2 hours and concentrated *in vacuo*. The residue was dried under high vacuum for one hour and dissolved in ethyl acetate (25 mL). The resulting solution was washed with saturated aqueous sodium bicarbonate solution, and the aqueous phase was extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, methanol/dichloromethane), and the desired product **16** (208 mg) was isolated and characterized by ^{1}H-NMR and LC/MS analysis (LRMS (MH) *m/z:* 496.64).

Example 7

[00209] Using the procedures set forth above, the following compounds were prepared:

Structure	**Calculated Molecular Mass**
	421.53206

	500.42812
	496.6433

	497.62802
	435.55864

Example 8

Monopolar Spindle Formation following Application of a KSP Inhibitor

[00210] Human tumor cells Skov-3 (ovarian) were plated in 96-well plates at densities of 4,000 cells per well, allowed to adhere for 24 hours, and treated with various concentrations of the compounds of the invention for 24 hours. Cells were fixed in 4% formaldehyde and stained with antitubulin antibodies (subsequently recognized using fluorescently-labeled secondary antibody) and Hoechst dye (which

stains DNA).

[00211] Visual inspection revealed that the compounds caused cell cycle arrest in the prometaphase stage of mitosis. DNA was condensed and spindle formation had initiated, but arrested cells uniformly displayed monopolar spindles, indicating that there was an inhibition of spindle pole body separation. Microinjection of anti-KSP antibodies also causes mitotic arrest with arrested cells displaying monopolar spindles.

Example 9

Inhibition of Cellular Proliferation in Tumor Cell Lines

[00212] Cells were plated in 96-well plates at densities from 1000-2500 cells/well of a 96-well plate and allowed to adhere/grow for 24 hours. They were then treated with various concentrations of drug for 48 hours. The time at which compounds are added is considered T_0. A tetrazolium-based assay using the reagent 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) (I.S> Patent No. 5,185,450) (see Promega product catalog #G3580, CellTiter 96® AQ$_{ueous}$ One Solution Cell Proliferation Assay) was used to determine the number of viable cells at T_0 and the number of cells remaining after 48 hours compound exposure. The number of cells remaining after 48 hours was compared to the number of viable cells at the time of drug addition, allowing for calculation of growth inhibition.

[00213] The growth over 48 hours of cells in control wells that had been treated with vehicle only (0.25% DMSO) is considered 100% growth and the growth of cells in wells with compounds is compared to this. KSP inhibitors inhibited cell proliferation in human ovarian tumor cell lines (SKOV-3).

[00214] A Gi_{50} was calculated by plotting the concentration of compound in μM vs the percentage of cell growth of cell growth in treated wells. The Gi_{50} calculated for the compounds is the estimated concentration at which growth is inhibited by 50% compared to control, i.e., the concentration at which:

$$100 \times [(\text{Treated}_{48} - T_0) / (\text{Control}_{48} - T_0)] = 50.$$

[00215] All concentrations of compounds are tested in duplicate and controls are averaged over 12 wells. A very similar 96-well plate layout and Gi_{50} calculation scheme is used by the National Cancer Institute (see Monks, et al., J. Natl. Cancer Inst. 83:757-766 (1991)). However, the method by which the National Cancer

Institute quantitates cell number does not use MTS, but instead employs alternative methods.

Example 10

Calculation of IC_{50}:

[00216] Measurement of a compound's IC_{50} for KSP activity uses an ATPase assay. The following solutions are used: Solution 1 consists of 3 mM phosphoenolpyruvate potassium salt (Sigma P-7127), 2 mM ATP (Sigma A-3377), 1 mM IDTT (Sigma D-9779), 5 μM paclitaxel (Sigma T-7402), 10 ppm antifoam 289 (Sigma A-8436), 25 mM Pipes/KOH pH 6.8 (Sigma P6757), 2 mM MgC12 (VWR JT400301), and 1 mM EGTA (Sigma E3889). Solution 2 consists of 1 mM NADH (Sigma N8129), 0.2 mg/ml BSA (Sigma A7906), pyruvate kinase 7U/ml, L-lactate dehydrogenase 10 U/ml (Sigma P0294), 100 nM KSP motor domain, 50 μg/ml microtubules, 1 mM DTT (Sigma D9779), 5 μM paclitaxel (Sigma T-7402), 10 ppm antifoam 289 (Sigma A-8436), 25 mM Pipes/KOH pH 6.8 (Sigma P6757), 2 mM MgC12 (VWR JT4003-01), and 1 mM EGTA (Sigma E3889). Serial dilutions (8-12 two-fold dilutions) of the compound are made in a 96-well microtiter plate (Corning Costar 3695) using Solution 1. Following serial dilution each well has 50 μl of Solution 1. The reaction is started by adding 50 μl of solution 2 to each well. This may be done with a multichannel pipettor either manually or with automated liquid handling devices. The microtiter plate is then transferred to a microplate absorbance reader and multiple absorbance readings at 340 nm are taken for each well in a kinetic mode. The observed rate of change, which is proportional to the ATPase rate, is then plotted as a function of the compound concentration. For a standard IC_{50} determination the data acquired is fit by the following four parameter equation using a nonlinear fitting program (e.g., Grafit 4):

$$y = \frac{\text{Range}}{1 + \left(\frac{x}{IC_{50}}\right)^{s}} + \text{Background}$$

where y is the observed rate and x the compound concentration.

[00217] Other compounds of this class were found to inhibit cell proliferation, although GI_{50} values varied. GI_{50} values for the compounds tested ranged from 200

nM to greater than the highest concentration tested. By this we mean that although most of the compounds that inhibited KSP activity biochemically did inhibit cell proliferation, for some, at the highest concentration tested (generally about 20 μM), cell growth was inhibited less than 50%. Many of the compounds have GI_{50} values less than 10 μM, and several have GI_{50} values less than 1 μM. Anti-proliferative compounds that have been successfully applied in the clinic to treatment of cancer (cancer chemotherapeutics) have GI_{50}'s that vary greatly. For example, in A549 cells, paclitaxel GI_{50} is 4 nM, doxorubicin is 63 nM, 5-fluorouracil is 1 μM, and hydroxyurea is 500 μM (data provided by National Cancer Institute, Developmental Therapeutic Program, http://dtp.nci.nih.gov/). Therefore, compounds that inhibit cellular proliferation at virtually any concentration may be useful.